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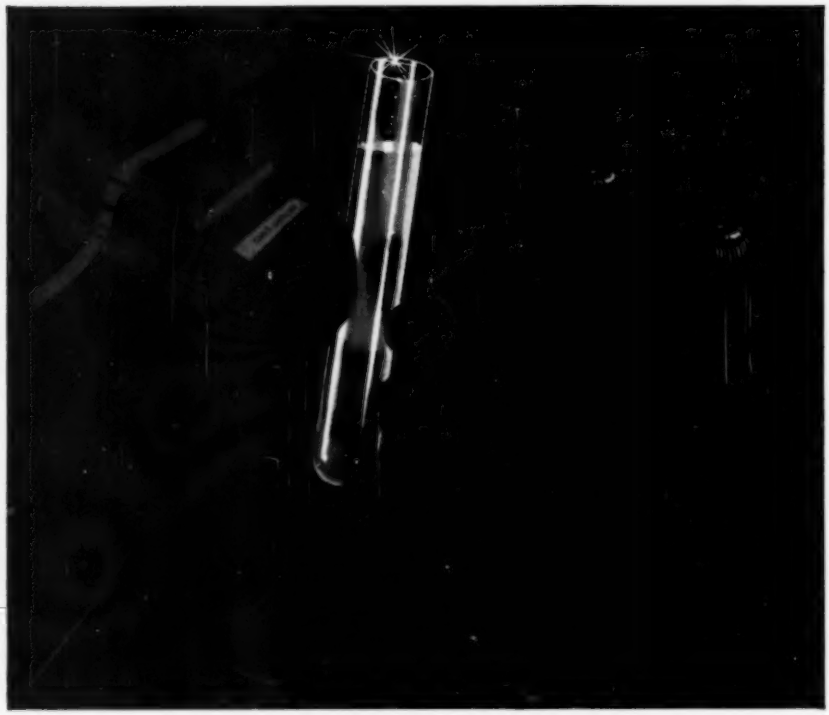

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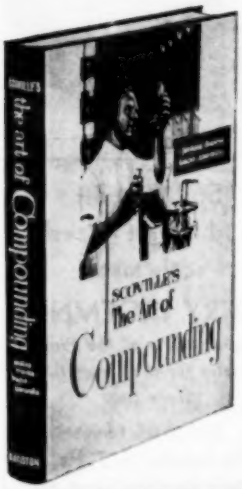

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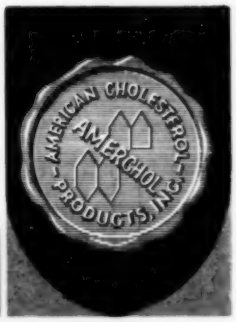

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VOLUME XLII — *AUGUST, 1953* — NUMBER 8

CONSECUTIVE NO. 16

p-Hydroxybenzoic Acid Esters as Preservatives

I. Uses, Antibacterial and Antifungal Studies, Properties and Determination*

By T. R. AALTO, M. C. FIRMAN, and N. E. RIGLER†

The history of the esters of *p*-hydroxybenzoic acid and their uses as preservatives in pharmaceuticals, cosmetics, foods, and industrial products are discussed. Antimicrobial studies show that the methyl, ethyl, propyl, and butyl esters are effective in low concentrations against fungi and Gram-positive bacteria, but less effective against Gram-negative bacteria. The esters are more fungistatic than fungicidal. The *p*-hydroxybenzoates were also tested against microorganisms in the presence of humans serum, and against the growth of *A. niger* in procaine penicillin suspensions. The physical and chemical properties of the *p*-hydroxybenzoates are extensively described, as well as methods of incorporation and a method of determination by ultraviolet absorption.

THE HISTORY of the practical application of the esters of *p*-hydroxybenzoic acid goes back to 1924, when Sabalitschka gave the first of his many reports on their use as antibacterial and antifungal agents (1). In the following years there appeared a great number of articles, mostly in European journals, on the properties and various uses of the esters, particularly as preservatives for pharmaceuticals, cosmetics, and foods. Interest spread to the United States, and commercial production was begun in the early 1930s.[1] The methyl, ethyl, propyl, and butyl esters have been studied most extensively, although benzyl-*p*-hydroxybenzoate and other esters and salts have also been available commercially.

The first official recognition of the parahydroxybenzoates came with the adoption of the methyl ester in the fifth edition (1934) of the *Swiss Pharmacopœia*, which now also includes the propyl ester. These two esters are currently listed in the supplement to the sixth edition of the *German Pharmacopœia*, the *British Pharmaceutical Codex*, which also includes the sodium salts, and in numerous other compendia. In the United States, the methyl ester was first adopted in the National Formulary VII and was subsequently admitted to the U. S. P. XIII, together with the propyl ester, identified, respectively, as Methylparaben and Propylparaben.

A wide variety of applications has been reported for these colorless, neutral preservatives. They have the additional attributes of being essentially nontoxic, nonvolatile, stable, and ef-

* Received December 22, 1952, from the Heyden Chemical Corporation, New York, N. Y.

† The data presented in this paper include the work of many members of the Heyden research, analytical, and control laboratories. The authors are particularly indebted to Max D. Blumenfeld for his work on the parahydroxybenzoates in cosmetics and to Irving B. Wakeman for the investigation of the stability of these esters and their determination.

[1] Methyl Parasept, Propyl Parasept, etc., trade mark of the Heyden Chemical Corporation.

fective in low concentrations against diverse microorganisms in acid, neutral, and alkaline solutions. The abundant literature has been reviewed from time to time, notably by MacDonald (2), Neidig and Burrell (3), Kossodo (4), and Sabalitschka (5). It is the purpose of this paper and subsequent articles to describe briefly the more important uses and potential uses of the parahydroxybenzoates, and to report on extensive studies of their properties, antibacterial and antifungal activity, pharmacology, toxicity, and effect on the skin. Some of the preliminary data were presented recently by Sokol (6).

APPLICATIONS

The history of these esters from the practical viewpoint of their usefulness can best be traced by citing the more important and recent literature pertaining to various fields of application. Space does not permit a complete or detailed review, but the investigations mentioned illustrate typical uses.

Pharmaceutical Preparations.—An attempt was made by Boehm (7, 8) and Suess (9) to reduce to table form the concentrations of the parahydroxybenzoic acid esters required to preserve many different pharmaceuticals and cosmetics. In general, higher concentrations were suggested in the case of oily products and emulsions than with aqueous solutions. In view of the greater activity of the higher esters, proportionately lower concentrations of them were recommended. There are so many factors involved, however—such as the physical form of the preparation, the nature of the ingredients, the *p*H, the method of manufacture, and the manner of use—that it is generally advisable to carry out suitable tests to determine optimum amounts.

Gershenfeld and Perlstein (11) investigated various preservatives for acid and basic type gelatins, which are useful as edible emulsifiers. The addition of 0.15% ethyl *p*-hydroxybenzoate effectively inhibited growth of typical resistant organisms in both acid (*p*H 3–4) and alkaline (*p*H 7–8) gelatin, illustrating the preservative's relative independence from *p*H. The same concentration of the propyl and butyl esters was tested with the acid gelatin and found effective, while the methyl ester was partially effective. Since all of the 0.15% of the ethyl, propyl, and butyl esters did not dissolve, as indicated by precipitation, the investigators concluded that preservation is actually attained with lower concentrations.

Preservatives for nasal jellies (1.5% tragacanth in water) were evaluated by Gershenfeld and Tomkin (12). Similar tests were run with ophthalmic solutions (2% boric acid, 1% paredrine hydrobromide). Subcultures were made after six, twenty, and thirty-five days. The ethyl (0.05%) and propyl (0.05%) esters, dissolved as the sodium salts, and the butyl ester (0.02%) exerted bactericidal action after twenty days. The methyl ester (0.15%), as the sodium salt, killed after thirty-five days. Efficient preservation, without objectionable color, appearance, or effect on collapsible tubes, was obtained with 0.05% ethyl, 0.03% propyl, or 0.02% butyl *p*-hydroxybenzoate, dissolved as the sodium salt in the case of the first two.

The value of combinations of esters, from the viewpoint of effectiveness and also solubility, was recognized by early investigators. In practice, two esters were generally used where increased activity and higher concentrations were needed. More recently, Borrini (10) compared the bactericidal efficiencies of phenol, methyl *p*-hydroxybenzoate, and propyl *p*-hydroxybenzoate with three different combinations of three or more esters. He inoculated aqueous solutions containing 0.05 to 0.25% bactericide with *S. aureus* and then seeded meat broth agar plates with these solutions after exposure times of five, ten, thirty and sixty minutes, and twenty-four hours. The growth on these plates diminished with increasing concentration of bactericide and time of exposure. Only five minutes were required with 0.25% of two different combinations to completely eliminate growth. The results showed that combinations of three or more esters were more active than an equal amount of a lower ester alone and also were more soluble. It has been demonstrated that the esters do not diminish each other's solubility in water.

In an investigation of therapeutic bases and the *p*H of the human skin, Harry (13) found a combination of 0.15% methyl, 0.05% propyl, and 0.02% butyl *p*-hydroxybenzoate in creams and lotions highly effective against a wide variety of microorganisms. The relatively low concentrations of 0.025% Methylparaben and 0.015% Propylparaben are prescribed in the U. S. Pharmacopeia for hydrophilic ointment (14). Darlington and Guth (15), in describing the properties of bentonite as a component of ointment bases, stated that mold growth in such bases is prevented by the incorporation of 0.15% Methylparaben and 0.05% Propylparaben. Sander, in a patent on a spermicidal composition (16), suggested the use of propyl *p*-hydroxybenzoate in combination with one or more antiseptic agents as an antimold ingredient.

The stability and neutral reaction of the *p*-hydroxybenzoates have been found advantageous in the preservation of vitamin C solutions, for which a combination of 0.18% methyl ester and 0.02% propyl ester has been recommended (17). A diabetic syrup containing gums, saccharin, and methyl *p*-hydroxybenzoate as a preservative was found to be stable on storage by Woo and Huyck (18). H. Berry (19) suggested 0.04% of a 65:35 mixture of methyl and propyl esters to preserve ephedrine eye drops.

An interesting recent use of the *p*-hydroxybenzoates as antifungal agents in aqueous antibiotic preparations deserves mention. Johnson and Lerrigo (20) found that the methyl and propyl esters did not adversely affect the stability of crystalline sodium penicillin G in saline solution. This compatibility has been confirmed with similar studies (21) made with aqueous solutions of potassium penicillin G and aqueous suspensions of procaine penicillin. Samples containing 0.2% methyl or 0.04% propyl ester were compared with controls over several weeks storage at 25, 38, and 50°. Further tests, described later, with suspensions of procaine penicillin G for aqueous injection showed that 0.02% propyl ester in the water of dilution prevented the growth of *A. niger* over a

period of sixty-one days at 25°. A combination of 0.18% methyl ester with 0.02% propyl ester is now used in this antibiotic preparation. In this connection Dietmann (22) suggested the use of combination of 0.07% methyl ester and 0.03% propyl ester for the maintenance of sterility in pharmaceuticals which cannot be heated. This combination of esters dissolved in water has long been familiar in Germany as *aqua conservans* (23).

Cosmetic Products.—Preservatives suitable for use in cosmetics must meet much the same requirements as those used in pharmaceuticals. Color, odor, stability, effectiveness in slightly alkaline media, and reaction on the skin are important considerations. The esters of *p*-hydroxybenzoic acid possess the desired properties to a high degree and are widely used in cosmetics. The applications include shampoos, creams, lotions, oils, mucilages, pastes, jellies, gum solutions, and others. The antibacterial properties of *p*-hydroxybenzoates have also found application in deodorant creams and powders.

According to Boehm (7, 8) concentrations up to 1% methyl *p*-hydroxybenzoate (or alternatively 0.3% ethyl, 0.2% propyl, or 0.15% butyl) may be required in creams of high oil content. It is advantageous to use the higher esters, propyl and butyl, as the oil content increases, particularly in oils or oil solutions. In emulsions and aqueous preparations, however, combinations of the methyl or ethyl ester with the higher esters are advisable. Such combinations also make it possible to readily incorporate higher concentrations of preservatives, which may occasionally be needed, without exceeding the solubilities of the esters. Experience indicates that better distribution is obtained by incorporating at least a part of the preservative in the aqueous phase.

Many papers have been published, some general and some specific, on the use of *p*-hydroxybenzoates in cosmetics (8, 24). Their use in dentifrices is frequently mentioned (25). Burlinson (26) found 0.05% of the propyl ester to be a most suitable preservative for a gum tragacanth mucilage. Deakers (27) recommended the methyl ester for gums used in facial masks. For hand lotions (28), he proposed a combination of 0.12% methyl ester dissolved in the aqueous phase and 0.10% propyl ester in the oil phase.

Of interest in the manufacture of oily products is the recommendation by Gorchoff (29) that the propyl ester be used as an antioxidant for vegetable oils in hand lotions. Recent tests with lard, by the active oxygen method, have confirmed the fact that this ester has appreciable antioxidant activity. Prevention of rancidity and preservation of a cold cream were described by Gottfried (30), who suggested 0.25% of the propyl ester or, alternatively, 0.1% propyl ester plus 0.12% methyl ester. Similar combinations were suggested for hair oils, skin ointments, aqueous starch pastes, and gum mucilages.

According to McLachlan (31), *p*-hydroxybenzoates have largely superseded older preservatives such as benzoic and salicylic acids. For the microbiological testing of cosmetics, he suggested microscopic examination of samples as well as inspection of incubated samples seeded with two bacteria, a yeast and a mold.

The effectiveness of various concentrations of *p*-hydroxybenzoates as preservatives for typical cosmetic products has been investigated (32). Samples of lotions and creams containing 0.02 and 0.10% of each ester (0.05 and 0.20% in the case of methyl ester) were inoculated with pure cultures of four bacteria, a yeast, and five fungi, and incubated at 28° for two weeks, or three months in the case of the fungi. The tests included a combination of equal parts of the methyl and propyl esters. The creams proved to have a natural resistance to the growth of the microorganisms used, and the growth of the fungi in a pancake make-up was inhibited by 0.5% of any of the esters. The results with the Karaya gum wave set lotion and mineral oil and stearic acid hand lotions indicated that good protection against the organisms tested is achieved with 0.1 to 0.2% of the preservative, particularly with combinations of the lower and higher esters. Products with different compositions may require higher concentrations, which are readily obtained with combinations of two or more esters.

Food Products.—The use of the esters of *p*-hydroxybenzoic acid as food preservatives was one of their most important early applications in Europe. Regulations permitting their use in specified foods were enacted in the years 1932 to 1938 in Germany, Hungary, Italy, Norway, Yugoslavia, Rumania, and Czechoslovakia (4). In Germany, where the *p*-hydroxybenzoates are currently in wide use, the regulations of 1932 permitted the ethyl and propyl esters or their sodium salts (in mixture or in combination with certain other materials such as salt or citric acid) in prescribed amounts in many different foods (33). Permissible concentrations included 0.08% in margarine, 0.09% in fruit preparations, 0.05% in nonalcoholic drinks, and 0.12% in confectionery.

Another food use of the *p*-hydroxybenzoates mentioned recently in the literature is in the prevention of mold growth on cheese. Anderson (34) suggested treatment with the methyl ester prior to coating with wax. Tammisto and Peltola (35) inhibited mold growth during curing by dipping the cheeses in a solution of the ester. The use of the esters on wrapping materials to prevent mold on the surface of foods has been suggested.

A critical investigation of preservatives for foods has been reported by von Schelhorn (36). He suggested the careful selection of a preservative, or a combination of two, so suit particular conditions of *p*H, composition, solubility, etc., so that a minimum concentration could be used. For instance, acids such as salicylic, benzoic, *p*-chlorobenzoic, formic, and propionic showed strong inhibition of certain microorganisms in strongly acid solutions, in which they were undissociated. In more neutral solutions, however, amounts up to 4 or 8% were necessary to achieve inhibition. His tests with various yeasts, fungi, and bacteria showed that ethyl *p*-hydroxybenzoate, on the other hand, was active in both acid and neutral media.

In discussing problems arising from the use of chemicals in food in England, Banfield (37) considered the *p*-hydroxybenzoic acid esters to hold out considerable promise as preservatives, in view of their effectiveness at neutral *p*H. The data available on their toxicity have been favorable, and if substantiated, there appeared to be potential benefits to both the manufacturer and the consumer in the use of these preservatives.

In the United States, the use of the *p*-hydroxybenzoates in foods, up to the present time, has been limited to a few minor specialty applications. Wider use was not encouraged because, in the opinion of Lehman (38), further data were necessary before their safety could be evaluated. The investigations designed to furnish additional data will be reported in succeeding articles.

Medicinal Uses.—The esters of *p*-hydroxybenzoic acid have been suggested for use in antimycotic therapy. Earlier work in this field includes the use by Lomholt (39) of a 5% solution of the methyl ester in alcohol in successfully treating epidermophytosis of the feet. Subsequently, Bang (40) used a similar solution in 70% alcohol and a salve containing 5% of the methyl ester to treat trichophytosis of the scalp. According to Sabalitschka (5), the official Danish list of galenicals includes a solution, a powder, and two ointments containing *p*-hydroxybenzoates, which are used in the treatment of mycoses.

In an investigation of epidermophytosis of the feet and hands, Loos (41) found that 0.01% of the propyl ester and 0.014% of the methyl and ethyl esters inhibited the growth of *Epidermophyton interdigitale*, and that 1% of these esters killed the fungus in thirty minutes. The fungistatic concentration for the sodium salts of the methyl and propyl esters was 0.02% with *Trichophyton gypseum asteroides*, and 0.02 and 0.01%, respectively, with *Microsporon audonini*. In clinical use, a salve containing 5% propyl ester and 10% salicylic acid successfully controlled infections due to these fungi. A powder containing 2 to 4% methyl ester and talc was recommended for prophylactic use.

More recently, Delpiana and Ugazio (42) reported on the treatment of numerous cases of mycoses of the hands and feet with ointments and solutions of *p*-hydroxybenzoates. Favorable results were obtained with 6% solution of the butyl ester in peanut oil, an ointment containing 10% of a mixture of esters and a 20% solution in alcohol of a mixture. The solution form (in alcohol or propylene glycol) was found to be most effective, and could be reduced in concentration in case of irritation.

A new and particularly interesting use of the *p*-hydroxybenzoates was described by McVay and Sprunt (43) in reporting on recent studies of moniliasis in broad spectrum antibiotic therapy. The *in vitro* tests with an 80:20 mixture of the methyl and propyl esters established their inhibitory action against five strains of yeast, including *C. albicans* and *C. krusei*, isolated from patients. Studies made with 186 patients being treated with aureomycin showed that the mixture of esters was of value in preventing the development of moniliasis. This was particularly true in the case of oral administration, even with the relatively small dose of 0.8 to 1.42 Gm. of *p*-hydroxybenzoates per day. Similar results were obtained with vaginal and rectal administration, the mixture of esters preventing the overgrowth of *C. albicans*. No toxic effects of the *p*-hydroxybenzoates were observed in these studies.

Miscellaneous Uses.—A number of industrial applications of *p*-hydroxybenzoates have been described in the literature, some of them involving combinations with other antimicrobial agents. Gelatin for photographic use can be preserved with a combination of the methyl and propyl esters, as can various types of emulsions. The esters have long found use in shoe dressings and in cutting oils. They have been found well suited for use as preservatives in pastes and glues that come into contact with the skin or with food products, and have aroused interest as antimold agents in food wrappings. The ethyl ester, in particular, has been employed as an intermediate in the synthesis of pharmaceuticals.

ANTIBACTERIAL AND ANTIFUNGAL STUDIES

Sabalitschka (5) recently reported the activity of the *p*-hydroxybenzoates in inhibition of a yeast fermentation as methyl 3.7, ethyl 5.3, propyl 25, butyl 40, and benzyl 69, when compared with phenol as 1. A study of the data compiled from various sources shows that in general the esters are more active against fungi than against bacteria and that higher esters are more active than the lower ones, although many exceptions to the latter statement are noted in the literature. For example Back (44) stated that, generally speaking, the methyl ester is more effective against mold, whereas the propyl ester is more effective against yeast.

In view of the discrepancies in the values reported for the antimicrobial activities of the various esters of *p*-hydroxybenzoic acid, it was concluded that it would be of value to determine the activities of a number of the commonly used esters, each against a variety of organisms, using standardized conditions. The results provide an antimicrobial spectrum of the esters. It is, of course, possible that appreciably different values might be obtained through variations in technique and strain of organism. By way of example, Gialdi and Ponci (45) reported inhibition of *S. aureus* and *E. coli* by 0.023% butyl *p*-hydroxybenzoate, values not in close agreement with those obtained in the present study.

Vincent and his coworkers (46–48) studied the inhibitory action of a number of esters of 4-hydroxybenzoic acid and related compounds against three fungi: *Aspergillus niger*, *Byssochlamya fulva*, and *Penicillium roqueforti*. They grew the fungi on agar plates made up with Czapek-Dex medium with sucrose and adjusted to *p*H 4.4 to 4.8. The diameter of the colony was measured at appropriate time intervals to provide for calculation of the dosage-response relationship. Values for log I_{50} ($-\log$ millimolar concentration giving 50% inhibition of growth rate) were reported for the esters methyl through heptyl and for a number of related compounds. Although there was some difference in the effect against the three organisms, there was obtained an approximately straight line relationship when log I_{50} was plotted against chain length of the ester. There were also straight lines when log water solubility and partition coefficient were plotted against chain length. Thus, the increasing inhibitory activities of the esters were closely correlated with decreasing solubility in water. The branched chain esters were slightly less active than the corresponding straight chain esters. The logarithmic dosage-response curves for all the esters had the same slope, pointing to a similar mode of action for all the esters.

In the determination of the inhibitory actions of the esters, it was decided to determine the concentration necessary to prevent growth. It is the consensus of those who work with antifungal agents that the concentration required to cause an inhibition of 50% of growth (or to prevent 50% of spores from germinating) can be more reliably determined than the concentration providing 100% inhibition (*cf.* articles by Vincent and coworkers). Nevertheless, it was considered advisable in this study to adhere to the older technique since determination of the absolute amounts required to prevent growth of organisms should be more directly translatable to practice in the pharmaceutical and food industries.

Experimental.—In the investigation of the antifungal activity of the *p*-hydroxybenzoates, the samples were dissolved in methanol at such concentrations that, when 0.5 cc. was added to the 25-cc. broth, the flasks contained the concentration shown. Twofold increments were generally used.

The medium was made up as follows: honey 85 Gm., peptone 10 Gm., water 1 L. The broth was dispensed into 125-cc. Erlenmeyer flasks, and the flasks were plugged and autoclaved for twenty minutes at 20 lb. pressure. A blank of inoculated medium and a control of 0.5-cc. methanol were run. Each flask was inoculated with one drop of spore suspension made by adding 25 cc. of sterile water to a well grown-out slant of the organism. Each concentration of each compound was run in triplicate. The flasks were allowed to stand for seven days. At the end of this time the quantity of growth was read, calling the growth in the blank flask 4+.

In making antibacterial tests, the plate streak method was used. The dilutions of the sample were made in water, using sufficient sodium hydroxide for solubility. The medium was so prepared that, when 5.0 cc. of the sample was added to 10 cc. of medium, the proper concentration of *p*-hydroxybenzoates and ingredients was obtained.

The medium was composed of glucose 22.5 Gm., peptone 15.0 Gm., sodium chloride 2.25 Gm., beef extract 4.5 Gm., agar 22.5 Gm., and water 1 L. The *p*H was approximately 6.8. The medium was dispensed into test tubes and sterilized. After addition of the samples, the contents of the tubes were poured into plates. Inoculations were made by dipping a loop in the suspension from 10 cc. of water on a twenty-four-hour slant of the organism and streaking on one sector on the plate. Readings were made after twenty-four hours.

The concentrations of methyl, ethyl, propyl, and butyl esters required to inhibit growth of a number of bacteria and fungi are given in Table I. Under the conditions used, the higher esters particularly are readily inhibitory to fungi in the concentrations which are feasible in pharmaceutical products. Some of the Gram-negative bacteria, such as *Pseudomonas aeruginosa* ATCC 9027 and *Pseudomonas fluorescens* ATCC 9721, are much more difficult to inhibit. Their growth was stopped by 0.2 to 0.4% of the methyl or ethyl ester, but 0.4 to 0.8%, far in excess of the solubility, was required with the propyl and butyl esters. The reason for this behavior has not been determined. It was found, however, that a combination of the four esters in the ratio of their solubilities in water at 25° inhibited the growth of these organisms at concentrations of 0.25 to 0.49%, which are below the saturation values.

In Table II are presented the comparative activities of a number of esters of *p*-hydroxybenzoic acid against *Aspergillus niger*. This organism was found to be the most resistant of all the fungi tested to the action of antimicrobial agents. The optimum chain length of the ester seems to be about three or four, as lower esters are more soluble but less active and the higher ones are so insoluble that a sufficient quantity for inhibition is difficult to get into water solution.

TABLE I.—ANTIMICROBIAL ACTIVITIES OF *p*-HYDROXYBENZOATES[a]

Microorganisms	Required for Inhibition, %			
	Methyl	Ethyl	Propyl	Butyl
Aspergillus niger ATCC 10254	0.1	0.04	0.02	0.02
Penicillium digitatum ATCC 10030	0.05	0.025	0.0063	<0.0032
Rhizopus nigricans ATCC 6227A	0.05	0.025	0.0125	0.0063
Trichoderma lignorum ATCC 8678	0.025	0.013	0.0125	0.0063
Chaetomium globosum ATCC 6205	0.05	0.025	0.0063	<0.0032
Trichophyton mentagrophytes ATCC 9533	0.016	0.008	0.004	0.002
Trichophyton rubrum ATCC 10218	0.016	0.008	0.004	0.002
Candida albicans ATCC 10231	0.1	0.1	0.0125	0.0125
Saccharomyces cerevisiae ATCC 9763	0.1	0.05	0.0125	0.0063
Saccharomyces pastorianus ATCC 2366	0.1	0.05	0.0125	0.0063
Bacillus subtilis ATCC 6633	0.2	0.1	0.025	0.0125
Bacillus cereus var. *mycoides* ATCC 6462	0.2	0.1	0.0125	0.0063
Staphylococcus aureus (*Micrococcus pyogenes* var. *aureus*) ATCC 6538P	0.4	0.1	0.05	0.0125
Sarcina lutea	0.4	0.1	0.05	0.0125
Klebsiella pneumoniae ATCC 10031	0.1	0.05	0.025	0.0125
Escherichia coli ATCC 9637	0.2	0.1	0.1	0.4
Salmonella typhosa	0.2	0.1	0.1	0.1
Salmonella shottmuelleri	0.2	0.1	0.05	0.1
Proteus vulgaris ATCC 8427	0.2	0.1	0.05	0.05
Aerobacter aerogenes ATCC 8308	0.2	0.1	0.1	0.4

[a] Precipitation was noted in those instances where the concentration used appreciably exceeded the water solubility of the ester.

TABLE II.—MINIMAL CONCENTRATION OF ESTERS OF *p*-HYDROXYBENZOIC ACID REQUIRED TO INHIBIT *A. niger*

	%
Methyl	0.1
Ethyl	0.04
Propyl	0.02
Isopropyl	0.05
Butyl	0.02
Isoamyl	>0.015–<0.1
Hexyl	>0.015–<0.1
Cyclohexyl	>0.015–<0.1
2-Ethylhexyl	>0.1
o-Chlorobenzyl	>0.1
8-Hydroxyethyl	>0.1
Tetra-hydrofurfuryl	>0.1
p-Chlorobenzyl	Insoluble
Glyceryl	>0.1
Pentaerythrityl	>0.1

Experiments on the effect of mixtures of methyl and propyl *p*-hydroxybenzoates on the growth of *A. niger* showed that the effects are additive. The data in Table III demonstrate that a mixture containing methyl and propyl esters, each at half the level causing complete inhibition, prevents growth of *A. niger*. This confirms the value of combinations where higher concentrations are required.

TABLE III.—ADDITIVE EFFECT OF *p*-HYDROXYBENZOATES

Methyl, %	Propyl, %	Amount of Growth
0.05	...	0
0.025	...	3+
...	0.015	0
...	0.01	+
...	0.005	3+
0.05	0.005	0
0.025	0.01	0
0.025	0.005	+

In Table IV is shown the relative effectiveness of propyl *p*-hydroxybenzoate, phenol, and sodium benzoate against five bacteria in a medium of *p*H 6.8 and *A. niger* in a medium of *p*H 5.2. It is notable that, whereas the bacteria differ in their resistance to the propyl ester and sodium benzoate, all were inhibited by 0.2% phenol but not by 0.1%.

TABLE IV.—COMPARATIVE EFFECTIVENESS OF PROPYL *p*-HYDROXYBENZOATE PHENOL, AND SODIUM BENZOATE

	Propyl *p*-Hydroxybenzoate, %	Phenol, %	Sodium Benzoate, %
B. subtilis	0.025	0.2	0.2
S. aureus	0.05	0.2	0.4
K. pneumoniae	0.025	0.2	0.2
E. coli	0.1	0.2	0.4
S. typhosa	0.1	0.2	0.2
A. niger	0.02	>0.04	>0.04

Over the *p*H range of 4 to 7 there was no difference in the effectiveness of the four individual *p*-hydroxybenzoates, methyl, ethyl, propyl, and butyl, against *A. niger*. At *p*H 8 there was evidence that growth was slightly greater at subinhibitory levels in the case of the propyl and butyl. It is believed that this has no significance for these two esters as contrasted with the other two. It is logical to expect a diminution in effectiveness of the compounds as the *p*H of the solution approaches the *p*K value of the acids. It is undoubtedly true that the undissociated molecule is more active than the ion. Hence, for example, at a *p*H identical with the *p*K of the *p*-hydroxybenzoate (about 8.5) the concentration of the undissociated acid is only half the total concentration, and some decrease in effectiveness would be expected. The esters of *p*-hydroxybenzoic acid thus differ from benzoic acid in being relatively independent of *p*H. Benzoic acid, with *p*K value of 4.2, cannot be expected to be active much above a *p*H of 4.

In 1911 Boeseken and Waterman (49) showed that *p*-hydroxybenzoic acid was utilized by molds as a nutrient. More recently Davis (50) has reported *p*-hydroxybenzoic acid to be a bacterial vitamin in that a mutant of *E. coli* unable to synthesize aromatic acids responded to addition of *p*-hydroxybenzoic acid. The latter was therefore suggested as an intermediate in the synthesis of aromatic compounds from shikimic acid. Davis also proposed *p*-hydroxybenzoic acid as a model for chemotherapeutic analogs selectively toxic to microorganisms. Accordingly, an experiment was set up to see whether the activity of ethyl *p*-hydroxybenzoate against *E. coli* would be reversed by the addition of *p*-hydroxybenzoic acid. The organism was inhibited by 0.1% of the ethyl ester, whereas there was growth at 0.05%. The presence of *p*-hydroxybenzoic acid at 0, 0.001, and 0.01% did not alter the inhibitory effect.

The activity of propyl *p*-hydroxybenzoate in the presence of serum was determined by an agar streak test. Human serum was incorporated into the agar at a 10% level. Concentrations were so arranged that the final plate contained the agar at its normal level, serum at 10%, and the ester at 0.8, 0.4, 0.2, 0.1, 0.05, and 0.025%. The results in per cent required to inhibit are given in Table V.

TABLE V.—EFFECT OF SERUM ON ACTIVITY OF PROPYL *p*-HYDROXYBENZOATE

	Control	Serum
B. subtilis	0.1	0.2
S. aureus	0.05	0.2
K. pneumoniae	0.025	0.1
E. coli	0.2	0.2
S. typhosa	0.2	0.2

Preservation of Antibiotic Solutions and Suspensions.—The esters of *p*-hydroxybenzoic acid were tested for preservative action in aqueous suspensions of procaine penicillin. Vials of procaine penicillin for aqueous injection 300,000 u/vial were injected with 5 cc. of solution of esters of *p*-hydroxybenzoic acid of the concentration shown in Table VI, followed by one drop of a suspension of spores of *A. niger*. The vials were allowed to stand at 25° for the intervals indicated and were examined macroscopically and microscopically for evidence of growth.

TABLE VI.—PRESERVATION OF PENICILLIN SUSPENSIONS WITH *p*-HYDROXYBENZOATES

Days	Methyl, % 0.01	Methyl, % 0.05	Propyl, % 0.01	Propyl, % 0.02	Propyl, % 0.04	No Preservative
12	+[a]	+	+	0	0	4+
21	4+	4+	+	0	0	4+
28	4+	4+	+	0	0	4+
42	4+	4+	+	0	0	4+
61	4+	4+	+	0	0	4+

[a] + is the smallest amount of growth and 4+ the greatest.

Samples of penicillin procaine for aqueous injection in cartridges were made up with a mixture of 0.07% methyl and 0.03% propyl *p*-hydroxybenzoate. Each cartridge was inoculated with one drop of a suspension of spores of *A. niger*. The control without preservatives showed scanty growth of the organism at eight days and abundant growth at fifteen days and subsequent intervals at 25°. There was no growth in the suspension containing the mixture of methyl and propyl *p*-hydroxybenzoate, even after fifty-five days.

PHYSICAL AND CHEMICAL PROPERTIES

The physical properties and solubilities of methyl, ethyl, propyl, and butyl esters of *p*-hydroxybenzoic acid are given in Table VII.

The methyl ester of *p*-hydroxybenzoic acid has a slight, characteristic odor, but the other esters are practically odorless in the usual, purified form. No particular taste is noticeable in solutions of the esters, but in saturated aqueous solution they exhibit a slight tang or weak burning sensation in the mouth. In terms of equal concentrations, it is most noticeable with the butyl ester. According to taste panel tests, this tang is not noticeable with lower concentrations or in the presence of flavorings or fatty materials.

The esters are stable in air and are resistant to hydrolysis in cold or hot water. According to laboratory studies (51), they show no hydrolysis under the usual conditions of sterilization. Aqueous solutions, buffered at a *p*H of 6, were heated for two hours at 100°, and thirty minutes at 120°, respectively. No hydrolysis could be detected by ultraviolet spectrophotometric analysis with an accuracy of about 2%. The same was true at *p*H 3. These heating periods produced a slight amount of hydrolysis at *p*H 8, amounting to about 6% with the propyl ester. In general, resistance to hydrolysis increased with the size of the alkyl side chain and with branching of the chain. In buffered solutions of *p*H 3, 7, and 8 at 25°, no hydrolysis was noted in six weeks, the duration of the test.

METHODS OF INCORPORATION

The incorporation of the *p*-hydroxybenzoic acid esters into various products requires a familiarity with their solubility characteristics, particularly in water. While their solubility in water is limited, they can be dissolved in aqueous products at room temperature provided good agitation is maintained. The rate of solution is a function of particle size. With the fine powders that are normally available, concentrations of 0.15% methyl, 0.10% ethyl, 0.03% propyl, or 0.015% butyl can be achieved in twenty or thirty minutes, according to laboratory tests. A coarse powder, on the other hand, dissolves only after long, continued stirring.

The esters can be dissolved more rapidly by heating the medium to 70 or 75° and stirring until solution is complete. This temperature should be kept about 20° below the melting point of the ester used, to prevent agglomeration of the particles. Other methods of incorporation involve the preparation of solutions of the esters in solvents. Alcohol is frequently used, and often one of the other ingredients, such as an oil or propylene glycol, will serve as a solvent. Such a solution must be added slowly with stirring to the aqueous phase, in order to prevent precipitation of the ester. The concentration in the solvent should not be over 15% with the methyl ester or 2.5% with the propyl to obtain clear solutions.

The resistance of the *p*-hydroxybenzoates to saponification makes it possible to dissolve them in 5% sodium hydroxide to form the soluble sodium salts, thus providing another means of incorporation. Only about 80% of the stoichiometric quantity of alkali is needed for solubilization, as for example, 4.20 lb. of 5% NaOH per pound of methyl *p*-hydroxybenzoate. Such solutions should be kept below 30° and used the same day as prepared. When used to give an ester concentration of 0.1%, they raise the *p*H of neutral, unbuffered products to about 9. In order to adjust the *p*H to 7, an amount of suitable acid, equivalent to the alkali used, should be added. This method of incorporation is equivalent to using the dry sodium salts of the esters, which have been prepared but have the disadvantages of caking and hydrolyzing in the presence of moisture.

METHODS OF DETECTION AND DETERMINATION

The literature contains a number of papers on the detection and determination of the *p*-hydroxybenzoates in various media (52-59). One of the simplest methods of detection and estimation is provided by Millon's reagent, which can be used on aqueous solutions in the absence of interfering substances. Prior extraction of the esters with ether or alcohol may be necessary. A more sensitive colorimetric method makes use of the reagent 4-aminoantipyrine, and has been developed for easy manipulation by Ettinger (60).

The *p*-hydroxybenzoates may be readily determined quantitatively in the absence of interfering substances by ultraviolet absorption. The transmission curves of the individual esters are virtually identical, with a broad absorption band at 245–270 mμ. The optical density is measured at 255 mμ, and the concentration of the ester can be calculated from a standard calibration curve. Interference from *p*-hydroxybenzoic acid, which has approximately the same molecular extinction coefficient as the ester in acid solution, may be eliminated by neutralizing the sample to *p*H 7, and then extracting the esters with ether. The ether is evaporated and the residue dissolved in warm water. *p*-Hydroxybenzoic acid and the ester may be determined in the presence of each other by measuring the optical density at *p*H 1 and *p*H 7, after first measuring molecular extinction coefficients of the

TABLE VII.—PROPERTIES OF THE *p*-HYDROXYBENZOIC ACID ESTERS

	Methyl	Ethyl	Propyl	Butyl
Molecular weight	152.14	166.17	180.20	194.23
Appearance	White powder	White powder	White powder	White powder
Melting point, ° C.	125–127°	116–118°	96–98°	69–71°
Solubility, Gm. per 100 Gm.				
Water				
25°	0.25	0.17	0.05	0.02
10°	0.20	0.07	0.025	0.005
80°	2	0.86	0.30	0.15
Methanol, 25°	59	115	124	220
Ethanol, 25°	52	70	95	210
Propylene glycol, 25°	22	25	26	110
Glycerine, 25°	1.7	ca. 0.5	0.4	ca. 0.3
Peanut oil, 25°	0.5	1	1.4	5
Acetone, 25°	64	84	105	240
Benzene, 25°	0.7	1.65	3	40
Ether, 25°	23	43	50	150
Carbon tetrachloride, 25°	0.1	0.9	0.8	1
Mineral oil, U. S. P., 25°	0.01	0.025	0.03	0.1

acid and the ester in the same buffer solutions.

p-Hydroxybenzoates in oils may be determined by dissolving the sample in petroleum ether and extracting the esters with three portions of 80% ethanol prior to measurement of the optical density.

The individual esters can be determined by employing a chromatographic separation and spectrophotometric estimation devised by Higuchi (61). This technique provides a means for analyzing mixtures of the esters and determining them in the presence of interfering substances.

SUMMARY

1. The history of the esters of *p*-hydroxybenzoic acid and their uses as preservatives in a wide variety of pharmaceuticals, cosmetics, foods, and industrial products, and also in antimycotic therapy are traced briefly, with references to pertinent literature.

2. Antimicrobial studies with 22 microorganisms show the methyl, ethyl, propyl, and butyl esters to be effective in low concentrations against fungi and Gram-positive bacteria and less effective against Gram-negative bacteria in the media used. The esters are more fungistatic than fungicidal, but their effect is additive, suggesting the use of combinations to achieve higher concentrations in water.

3. The *p*-hydroxybenzoates are substantially equally effective against microorganisms in acid or neutral solutions in the *p*H range of 4 to 8. Their antimicrobial activity is slightly lower in the presence of human serum.

4. The growth of *A. niger* in procaine penicillin suspensions is inhibited by 0.02% propyl ester of *p*-hydroxybenzoic acid. A mixture of 0.07% methyl and 0.03% propyl ester prevents spore growth in pencillin cartridges.

5. An extensive description of the physical and chemical properties of the *p*-hydroxybenzoates is given. They are stable and do not hydrolyze under the conditions of heat sterilization (two hours at 100° or one-half hour at 120°) at *p*H 3 to 8.

6. Methods of incorporation are discussed in detail and a method of determination by ultraviolet absorption is outlined.

REFERENCES

(1) Sabalitschka, T., *Chem. Ztg.*, **118**, 703(1924).
(2) MacDonald, E. M., THIS JOURNAL, *Pract. Pharm. Ed.*, **3**, 181(1942).
(3) Neidig, C. P., and Burrell, H., *Drug & Cosmetic Ind.*, **54**, 408(1944).
(4) Kossodo, M., Report to the Ministry of Public Health and Social Aid (Lima, Peru), 1949.
(5) Sabalitschka, T., *Oesterr. Apoth. Ztg.*, **3**, 458(1949).
(6) Sokol, H., *Drug Standards*, **20**, 89(1952).
(7) Boehm, E., and Jeglinski, H., *Pharm. Ztg.*, **73**, 30 (1928).
(8) Boehm, E., *Drug & Cosmetic Ind.*, **32**, 510(1933).
(9) Suess, A., *Am. Perfumer Essent. Oil Rev.*, **55**, 6 (1936).
(10) Borrini, T., *Boll. chim. farm.*, **89**, 483(1950).
(11) Gershenfeld, L., and Perlstein, D., *Am. J. Pharm.*, **3**, 277(1939).
(12) Gershenfeld, L., and Tomkin, D., *ibid.*, **11**, 385 (1939).
(13) Harry, R. G., *Brit. J. Dermatol. Syphilis*, **54**, 1(1942).
(14) "United States Pharmacopeia," Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950, p. 391.
(15) Darlington, R. C., and Guth, E. P., THIS JOURNAL, *Pract. Pharm. Ed.*, **11**, 82(1950).
(16) Sander, F. V., U. S. pat. 2,541,103 (1951).
(17) Anon., *Bull. Am. Soc. Hosp. Pharm.*, **6**, 118(1949).
(18) Woo, M., and Huyck, C. L., *Bull. Nat. Formulary Comm.*, **16**, 140(1948).
(19) Berry, H., *Pharm. J.*, **150**, 10(1943).
(20) Johnson, B., and Lerrigo, A. F., *Quart. J. Pharm. Pharmacol.*, **20**, 183(1947).
(21) Heyden Chemical Corp., Research Laboratories, Princeton, N. J., (Unpublished data).
(22) Dietmann, H., *Pharm. Ind.*, **12**, 205(1950).
(23) Dultz, G., *Deut. Apoth. Ztg.*, **55**, 752(1940).
(24) Augustin, J., *Drug & Cosmetic Ind.*, **34**, 516(1932).
(25) Anon., *ibid.*, **63**, 655(1948).
(26) Burlinson, H., *Quart. J. Pharm. Pharmacol.*, **7**, 489 (1934).
(27) Deakers, T., *Drug & Cosmetic Ind.*, **38**, 479(1934).
(28) Deakers, T., *ibid.*, **38**, 37(1936).
(29) Gorchoff, N. T., *ibid.*, **46**, 682(1940).
(30) Gottfried, D., *Perfumery Essent. Oil Record*, **37**, 93 (1946).

(31) McLachlan, T., *Soap, Perfumery & Cosmetics*, **19**, 166(1946).
(32) Heyden Chemical Corp., Research Laboratories, Garfield, N. J., (Unpublished data).
(33) Jacobs, M. B., "The Chemistry and Technology of Food and Food Products," Interscience Publishers, Inc., New York, Vol. III, 1951, p. 1939.
(34) Anderson, E. G., *Chemistry and Industry*, **11**, 195 (1950).
(35) Tammisto, E. S., and Peltola, E., *Proc. 12th Intern. Dairy Congr.* (Stockholm), **3**, 213(1949).
(36) von Schelhorn, M., *Deut. Lebensm. Rundschau*, **47**, 16(1951).
(37) Banfield, F. H., *Chemistry and Industry*, **2**, 114 (1952).
(38) Lehman, A. J., *Bull. Assoc. Food & Drug Officials U. S.*, **14**, 2(1950).
(39) Lomholt, S., *Med. Klin.*, **34**, 118(1938).
(40) Bang, H., *Dermatol. Wochschr.*, **105**, 1096(1937).
(41) Loos, H. O., *Arch. Dermatol., u. Syphilis*, **173**, 109 (1935).
(42) Delpiana, J., and Ugazio, D., *El dia Medico (Argentina)*, **21**, 10(1949).
(43) McVay L. B., and Sprunt, D. H., *Proc. Soc. Exptl. Biol. Med.*, **78**, 759(1952).
(44) Back, S., *Pharm. J.*, **128**, 328(1932).
(45) Gialdi, F., and Ponci, R., *Form. sci. e tec. (Pavia)*, **5**, 642(1950).
(46) Vincent, J. M., *J. Soc. Chem. Ind.*, **66**, 149(1947).
(47) Cavill, G. W. K., and Vincent, J. M., *ibid.*, **66**, 175 (1947).
(48) Cavill, G. W. K., and Vincent, J. M., *ibid.*, **68**, 12 (1949).
(49) Boeseken, J., and Waterman, H. J., *Verslag Kon. Akad. Wetenschap.*, Amsterdam, **20**, 552(1911).
(50) Davis, B. D., *Nature*, **166**, 1120(1950).
(51) Heyden Chemical Corporation, Research Laboratories, Garfield, N. J. (Unpublished data).
(52) Boyce, E. G., *J. Assoc. Official Agr. Chemists*, **28**, 301(1945).
(53) Emerson, J., *J. Org. Chem.*, **8**, 417(1943).
(54) Friend, J. A., *J. Proc. Roy. Soc. N. S. Wales*, **80**, 224(1947).
(55) Johnson, H. W., *Analyst*, **71**, 77(1946).
(56) Jones, C. N., *J. Assoc. Official Agr. Chemists*, **30**, 486(1947).
(57) Martin, R. W., *Anal. Chem.*, **21**, 1914(1949).
(58) Sabalitschka, T., *Congr. intern. tech. et chim. ind. agr. Compt. rend. 6e Congr. Budapest*, **1**, 519(1930).
(59) Sager, E. E., Schooley, M. R., Carr, A. S., and Acree, S. F., *J. Research Natl. Bur. Standards*, **35**, 521(1948).
(60) Ettinger, M. B., Ruchhoft, C. C., and Lishka, R. J., *Anal. Chem.*, **23**, 1783(1951).
(61) Higuchi, T., Patel, K. P., Bonow, E. R., and Landsman, J., THIS JOURNAL, **41**, 293(1952).

Antitubercular Substances. II. Substitution Products of Isonicotinic Hydrazide*

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A series of thirty N^2-substitution products of N^1-isonicotinyl hydrazine has been prepared and characterized. The majority of the substances had an *in vivo* antitubercular activity of the same order of magnitude as that of isonicotinic hydrazide. A short series of other compounds which can also be considered as derivatives of isonicotinic hydrazide has also been prepared and characterized but found to be devoid of any significant antitubercular action.

IN CONTINUATION of our search (1) for effective antitubercular substances we are now describing the synthesis of a number of derivatives of isonicotinic hydrazide, as well as a few other related compounds. In view of certain differences in the clinical behavior of isonicotinic hydrazide, its N^2-isopropyl derivatives and its N^2-glucosyl derivative (2–4) it seemed of interest to learn the effect of considerable variation in substituents in the parent molecule on the antitubercular activity of the substance.

In Table I are presented thirty substances in which N^2 of isonicotinic hydrazide is substituted by either an alkylidene or an alkyl group. If the hydrazones (i. e., the alkylidene compounds) are only active by virtue of their hydrolysis to the parent isonicotinic hydrazide none of them (Nos. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 26, 28, and 29) should be more active, on a molar basis than isonicotinic hydrazide. If, on the other hand, the alkylidene compounds act primarily *per se*, there should be some more or less constant relationship (dependent on the azomethine linkage) between the activities of the members of pairs composed of a hydrazone and its corresponding reduced compound, i. e., Nos. 1 and 2, 3 and 4, 5 and 6, 7 and 8, etc.

A comparison of Nos. 2 and 4 should show the effect of a methoxyl substituent and one of Nos. 1 and 25 should show the effect of a carbethoxyl substituent if the hydrazones have an action *per se*. Nos. 2 and 6 might disclose an effect due to an additional methylene group; Nos. 6 and 8 differ only in the position of the methyl group while No. 10 has the same carbon content but in a straight rather than branched chain. Nos. 9 and 10 might indicate the effect of extended conjugation while 10 and 12 differ in (straight) chain length. The addition of methyl groups to a butyl chain is shown in the series 10, 14, 16; 6 and 20 differ similarly. The effect of a strongly lipophilic group is to be seen in 22

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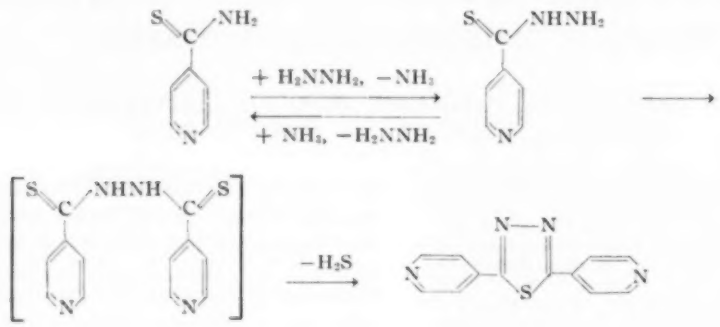
and even more so in 24. If oxidation at a secondary or tertiary carbon atom is of the importance in compounds of this type that it may be in antimalarials of the naphthoquinone type (5), this should be found in a comparison of Nos. 16 and 18. In the phenyl-substituted compounds, Nos. 27 and 30 differ by a methylene group and Nos. 28 and 29 by a methyl group.

These substances were evaluated for their action *in vitro* against *Mycobacterium tuberculosis*, strains $H_{37}R_v$ and D_4M_3, and by the drug-diet technique in mice infected with the virulent bovine D_4M_3 strain. Generally speaking, about ninety per cent of the substances had an activity *in vivo* of the order of magnitude of that of the parent isonicotinic hydrazide molecule. The answers to the questions which we raised concerning structure-activity relationships among these compounds can only come from a study of the ST_{50} and similar data which will be published elsewhere by other people from these laboratories.

of the parent molecule to N^1-isonicotinyl-N^2-acetylhydrazine lowers the activity considerably (7), dehydration of the acetyl compound to the oxadiazole, No. 34, removes completely any activity, just as does similar ring closure of the hypothetical thio-isonicotinyl acetyl hydrazine to the thiadiazole, No. 35, which is likewise inactive. We intended to prepare the sulfur analog of the parent molecule, thioisonicotinic hydrazide, by taking advantage of the equilibrium which can be set up between amides and hydrazines (and presumably between thioamides and hydrazines) but in our single attempt at the reaction it went too far and the thioisonicotinic hydrazide produced underwent the equilibrium interchange with a second thio-isonicotinic amide molecule to give the N^1,N^2-dithioisonicotinyl hydrazine which intramolecularly lost hydrogen sulfide under the reaction conditions to furnish the thiadiazole, No. 36, that also was inactive.



In Table II are presented a number of other compounds which may also be looked upon as derivatives, of a sort, of the parent isonicotinic hydrazide molecule. The amide, No. 31, may be considered as the counterpart of No. 2 in which an —NH— group has been omitted and it, just as the unsubstituted isonicotinamide (7) was devoid of any significant antitubercular activity. Activity was likewise destroyed by quaternization of the parent molecule to No. 33, or by the introduction of two methoxyl groups to give No. 32. The latter substance was prepared by hydrazinolysis of the corresponding ethyl ester, obtainable from the dimethoxyacid prepared by the procedure of Büchi, *et al.* (8), for higher 2,6-dialkoxy isonicotinic acids; it seemed of some interest that 2,6-dimethoxypyridine-4-carboxylic acid was obtained from either 2,6-dichloropyridine-4-carboxylic acid (9) or its methyl ester (10) on treatment with *dry* sodium methoxide in *dry* methanol. While acetylation

EXPERIMENTAL

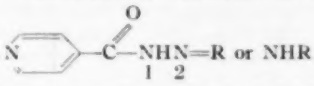
The isonicotinic hydrazones (the alkylidene compounds of Table I) were in general prepared by heating isonicotinic hydrazide at steam-bath temperature for an hour or two with two or more molar equivalents of the aldehyde or ketone, then stripping the excess carbonyl compound under vacuum and recrystallizing the cooled residue from an appropriate solvent. Accordingly, the following experimental details are given only in outline form except where there was particular deviation from the general procedure.

N^1-Isonicotinyl-N^2-(2-propylidene)-hydrazine, No. 1.—Four parts by weight of acetone, refluxed one hour, stripped, and residue recrystallized from ethanol-ligroin (b. p. 60–68°); quantitative yield.

N^1-Isonicotinyl-N^2-(1-methoxy-2-propylidene)-hydrazine, No. 3.—Two molar parts of methoxyacetone (11), refluxed one hour, stripped and crystalline residue, m. p. 94–97° (97% yield), washed with ether and recrystallized twice from $2^1/_2$ parts by weight of ethyl acetate.

N^1-Isonicotinyl-N^2-(2-butylidene)-hydrazine, No. 5.—Two molar parts of methyl ethyl ketone, two hours on the steam bath, stripped and crystalline

TABLE I.—N^1-ISONICOTINYL-N^2-SUBSTITUTED HYDRAZINES



No.	R	M. P., °C.	Empirical Formula	Analyses Calculated C	Calculated H	Found C	Found H
1.	$=CCH_3$ (CH₃)	164–164.7	$C_9H_{11}N_3O$	61.00	6.26	60.90	6.31
2.	$-CHCH_3$ (CH₃)	112.5–113.5	$C_9H_{13}N_3O$	60.31	7.31	60.05	7.35
3.	$=CCH_2OCH_3$ (CH₃)	110.5–111.5	$C_{10}H_{13}N_3O$	57.95	6.32	58.06	6.22
4.	$-CHCH_2OCH_3$ (CH₃)	72–76	$C_{10}H_{15}N_3O$	57.40	7.23	56.92	7.44
5.	$=CCH_2CH_3$ (CH₃)	95–96	$C_{10}H_{13}N_3O$	62.82	6.85	63.15	7.08
6.	$-CHCH_2CH_3$ (CH₃)	b. p. 144–148 (0.17 mm.)	$C_{10}H_{15}N_3O$	62.15	7.82	61.67	7.54
7.	$=CHCHCH_3$ (CH₃)	140–141	$C_{10}H_{13}N_3O$	62.82	6.85	62.87	6.64
8.	$-CH_2CHCH_3$ (CH₃)	b. p. 124–125 (0.04 mm.)	$C_{10}H_{15}N_3O$	62.15	7.82	62.28	8.05
9.	$=CHCH=CHCH_3$	209.5–210.5	$C_{10}H_{11}N_3O$	63.46	5.86	63.70	5.99
10.	$-CH_2CH_2CH_2CH_3$	65–68	$C_{10}H_{15}N_3O$	62.15	7.82	62.32	8.11
11.	$=CHC_6H_{13-n}$	101.7–102.5	$C_{13}H_{19}N_3O$	66.92	8.21	66.75	8.33
12.	$-CH_2C_5H_{13-n}$ (CH₃)	40–42	$C_{13}H_{21}N_3O$	66.35	9.00	66.49	8.92
13.	$=CHCH_2CHCH_3$ (CH₃)	118.5–119.5	$C_{11}H_{15}N_3O$	64.35	7.37	64.58	7.70
14.	$-CH_2CH_2CHCH_3$ (CH₂ CH₃)	b. p. 135–146 (10^{-3} mm.)	$C_{11}H_{17}N_3O$	63.74	8.27	63.54	8.38
15.	$=CCH_2CHCH_3$ (CH₃ CH₃)	93–94	$C_{12}H_{17}N_3O$	65.72	7.82	66.02	7.68
16.	$-CHCH_2CHCH_3$ (CH₂ CH₃)	88.5–91	$C_{12}H_{19}N_3O$	65.13	8.65	65.33	8.58
17.	$=CCH_2CCH_3$ (OH)	146.5–147.5	$C_{12}H_{17}N_3O_2$	61.23	7.28	61.52	7.61
18.	$-CHCH_2CCH_3$ (CH₃ OH CH₃)	83–86	$C_{12}H_{19}N_3O_2$	60.74	8.07	60.56	7.90
19.	$=C(CH_3)CCH_3$ (CH₃ CH₃)	120–121	$C_{12}H_{17}N_3O$	65.72	7.82	65.62	7.68
20.	$-CHCCH_3$ (CH₃ CH₃ CH₃)	95–97	$C_{12}H_{19}N_3O$	65.13	8.65	65.01	8.53
21.	$=CCH_2CHCH_2$ (CH₃ CH₃ CH₂CHCH₃)	128.5–129.5	$C_{15}H_{22}N_3O$	68.92	8.87	68.73	8.60

(*Table I.—Continued.*)

22.	$-CHCH_2CHCH_3$ CH_3	b. p. 154–164 (10^{-2} mm.)	$C_{15}H_{25}N_3O$	68.40	9.57	68.53	9.69
23.	$=CC_8H_{17-n}$ C_8H_{17-n}	20–25°; b. p. 158–208° (10^{-3} mm.)	$C_{23}H_{39}N_3O$	73.95	10.52	73.64	10.31
24.	$-CHC_8H_{17-n}$ CH_3	b. p. 120–130 (10^{-3} mm.)	$C_{22}H_{41}N_3O$	73.54	11.00	74.42	11.18
25.	$=CCH_2COOC_2H_5$ CH_3	95–96.5	$C_{12}H_{15}N_3O_3$	57.82	6.07	57.44	5.71
26.	$=CC_6H_5$[a] CH_3	174.5–175.2	$C_{14}H_{13}N_3O$	70.27	5.47	70.31	5.49
27.	$-CHC_6H_5$	96–97	$C_{14}H_{15}N_3O$	69.69	6.27	69.80	6.54
28.	$=CHCH_2C_6H_5$ CH_3	140–141	$C_{14}H_{13}N_3O$	70.27	5.47	70.20	5.23
29.	$=CHCHC_6H_5$ CH_3	128.5–130	$C_{15}H_{15}N_3O$	71.12	5.97	70.68	6.50
30.	$-CH_2CHC_6H_5$	b. p. 182–187 (10^{-3} mm.)	$C_{15}H_{17}N_3O$	70.56	6.71	70.34	6.62

[a] The *in vitro* activity and mouse tolerated dose of this compound were reported by Shchukina and co-workers (6) who gave its melting point as (160–161° for No. 1, *N* analysis satisfactory; 165–166° for No. 26, *N* analysis satisfactory).

[b] This is one of the substances used in the clinical studies reported in references (2–4), but it has not been described in the chemical literature.

[c] *In vitro* and *in vivo* activity of this material were described by Bernstein and co-workers (7) but no characterization of it is in the chemical literature.

residue, m. p. 95–96° (quantitative yield), recrystallized twice from benzene.

N^1-Isonicotinyl-N^2-(2-methylpropylidene)-hydrazine, No. 7.—Two molar parts isobutyraldehyde, one hour on steam-bath, crystallized on cooling; washed with ether and crystals, m. p. 139.5–140.5° (87% yield), recrystallized from benzene.

N^1-Isonicotinyl-N^2-(2-butenylidene)-hydrazine, No. 9.—One molar part crotonaldehyde and two volumes of benzene, one hour on steam bath, stripped and recrystallized twice from methanol.

N^1-Isonicotinyl-N^2-heptylidenehydrazine, No. 11.—Two molar parts *n*-heptaldehyde, one hour on steam bath and stripped; washed with ether and crystals, m. p. 100.5–102.5° (96% yield), recrystallized first from ethyl acetate and then from ethyl acetate-ligroin (b. p. 60–68°).

N^1-Isonicotinyl-N^2-(3-methylbutylidene)-hydrazine, No. 13.—Two molar parts isovaleraldehyde, one hour on steam bath, chilled, diluted with ether, and filtered; crystals, m. p. 115–118° (69% yield), recrystallized from benzene.

N^1-Isonicotinyl-N^2-(4-methyl-2-pentylidene)-hydrazine, No. 15.—Two molar parts methyl isobutyl ketone, two hours on the steam bath, stripped, and the crystalline residue, m. p. 95.5–96.5°, recrystallized from acetone.

N^1-Isonicotinyl-N^2-(4-hydroxy-4-methyl-2-pentylidene)-hydrazine, No. 17.—Two molar parts diacetone alcohol, two hours on the steam bath, stripped, residue washed with ether, and filtered; crystals, m. p. 135–136.5° (82% yield), recrystallized twice from acetone.

N^1-Isonicotinyl-N^2-(3,3-dimethyl-2-butylidene)-hydrazine, No. 19.—Two molar parts of pinacolone, refluxed five and one-half hours, stripped, residue washed with ether, and filtered; crystals, m. p. 118–119.5° (96% yield), recrystallized from benzene.

N^1-Isonicotinyl-N^2-(2,6-dimethyl-4-heptylidene)-hydrazine, No. 21.—Four molar parts diisobutyl ketone, refluxed three and one-half hours through a Dean-Starke water separator, stripped and residue washed with ether; recrystallized three times from methyl ethyl ketone-ligroin (b. p. 60–68°).

N^1-Isonicotinyl-N^2-(9-heptadecylidene)-hydrazine, No. 23.—Equimolar part of di-*n*-octyl ketone, heated three hours at 145–150°, cooled, diluted with ether, filtered, and filtrate fractionally distilled to give the product, n_D^{25} 1.5058.

N^1-Isonicotinyl-N^2-(1-carbethoxy-2-propylidene)-hydrazine, No. 25.—Equimolar part of ethyl acetoacetate and two volumes of ethanol, refluxed one hour, stripped, recrystallized from *n*-propanol (very soluble).

N^1-Isonicotinyl-N^2-(1-phenylethylidene)-hydrazine, No. 26.—To molar parts of acetophenone, heated one hour at 145°, cooled, rinsed with ether, and recrystallized three times from isopropanol.

N^1-Isonicotinyl-N^2-(2-phenylethylidene)-hydrazine, No. 28.—Phenylacetaldehyde (1.4 molar parts) heated three and one-half hours on the steam bath. The gummy reaction mixture was washed four times with ether and then was dissolved in ethyl acetate. One-fourth volume of ligroin (b. p. 60–68°) was added, the mixture was filtered, and the filtrate was refrigerated. The crystals obtained from the filtrate were recrystallized twice from ethyl acetate-ligroin (b. p. 60–68°).

N^1-Isonicotinyl-N^2-(2-phenylpropyli lene)-hydrazine, No. 29.—Equimolar amounts of hydratropic aldehyde, heated three hours on the steam bath, cooled, washed with ether, recrystallized twice from benzene.

The N^2-alkyl isonicotinic hydrazides were prepared by catalytic hydrogenation of the corresponding alkylidene compounds. The general

procedure was to place the alkylidene compound, Adams platinum oxide catalyst, and absolute alcohol in a hydrogenation bottle and reduce the compound on a Parr shaker at room temperature and atmospheric pressure, using a gas burette, for the number of hours required for the uptake of an amount of hydrogen equimolar with the amount of alkylidene compound used. The catalyst was then removed by filtration, the alcohol was stripped off under vacuum and the residue was either recrystallized from an appropriate solvent, usually with charcoaling, or distilled. Accordingly, experimental details are given in only outline form.

N^1-Isonicotinyl-N^2-(2-propyl)-hydrazine, No. 2.—Alkylidene compound (No. 1) (0.044 mole), 125 cc. ethanol, 0.25 Gm. catalyst, one hour for 1,111 cc. uptake (theory 1,080); recrystallized from 50:50 benzene-ligroin (b. p. 60–68°).

This compound could also be made by direct alkylation of isonicotinic hydrazide with isopropyl bromide.

To a fresh solution of sodium (2.3 Gm., 0.10 mole) in absolute alcohol (50 cc.), cooled to about 50°, there was added a solution of isonicotinic hydrazide (13.7 Gm., 0.10 mole) in absolute alcohol (150 cc.); the mixture was refluxed five minutes and cooled. There was then added isopropyl bromide (12.3 Gm., 0.10 mole) and the now reddish solution was refluxed three hours. The precipitated sodium bromide was removed by filtration, the filtrate was concentrated under vacuum, diluted with ether, and more sodium bromide removed. The alcoholic-ethereal filtrate was taken to a syrup, under vacuum, and refrigerated under ether for a week, during which time it changed to a mixture of brown gum and light-colored crystals (4.25 Gm., 28% yield). After several recrystallizations from 1:1 ethylene dichloride-ligroin (b. p. 60–68°), the material melted at 109.5–110.5°.

Anal.—Calcd. for $C_9H_{13}N_3O$: C, 60.31; H, 7.31. Found: C, 60.62; H, 7.26.

There was no depression in melting point when this material was admixed with a sample of material m. p. 112.5–113.5°, prepared by reduction of the isopropylidene derivative.

N^1-Isonicotinyl-N^2-(1-methoxy-2-propyl)-hydrazine, No. 4.—Alkylidene compound (No. 3) (0.13 mole), 100 cc. ethanol, 1.0 Gm. catalyst, three hours for 3,370 cc. uptake (theory = 3,180); distilled at 157–162° (0.001 mm.), redistilled at 144–149° (0.001 mm.), redistilled at 144–149° (0.002 mm.), and this distillate crystallized.

N^1-Isonicotinyl-N^2-(2-butyl)-hydrazine, No. 6.—Alkylidene compound (No. 5) (0.078 mole), 150 cc. ethanol, 0.5 Gm. catalyst, one and three-quarter hours for 2,000 cc. uptake (theory = 1,970); distilled at 132–142° (0.02 mm.) and then redistilled.

N^1-Isonicotinyl-N^2-isobutylhydrazine, No. 8.—Alkylidene compound (No. 7) (0.08 mole), 150 cc. ethanol, 0.5 Gm. catalyst, ten hours for 1,940 cc. uptake (theory = 1,960); distilled at 140° (0.005 mm.) and then redistilled.

N^1-Isonicotinyl-N^2-*n*-butylhydrazine, No. 10.—Alkylidene compound (No. 9) (0.077 mole), 150 cc. ethanol, 1.0 Gm. catalyst, three hours for 3,900 cc. uptake (theory = 3,800); recrystallized four times from 50:50 benzene-ligroin (b. p. 60–68°).

N^1-Isonicotinyl-N^2-*n*-heptylhydrazine, No. 12.—Alkylidene compound (No. 11) (0.026 mole), 100 cc. ethanol, 0.2 Gm. catalyst, three hours for 680 cc. uptake (theory = 670 cc.); distilled at 158–164° (0.04 mm.), distillate crystallized.

N^1-Isonicotinyl-N^2-(3-methylbutyl)-hydrazine, No. 14.—Alkylidene compound (No. 13) (0.077 mole), 125 cc. ethanol, 0.4 Gm. catalyst, three hours for 1,930 cc. uptake (theory = 1,970); distilled at 142–152° (0.001 mm.), then redistilled.

N^1-Isonicotinyl-N^2-(4-methyl-2-pentyl)-hydrazine, No. 16.—Alkylidene compound (No. 15) (0.023 mole), 50 cc. ethanol, 0.1 Gm. catalyst, one hour for 583 cc. uptake (theory = 560); recrystallized twice from 50:50 benzene-ligroin (b. p. 60–68°).

N^1-Isonicotinyl-N^2-(4-hydroxy-4-methyl-2-pentyl)-hydrazine, No. 18.—Alkylidene compound (No. 17) (0.052 mole), 150 cc. ethanol, 0.4 Gm. catalyst, nine and one-half hours for 1,340 cc. uptake (theory = 1,370); recrystallized four times from benzene-ligroin (b. p. 60–68°).

N^1-Isonicotinyl-N^2-(3,3-dimethyl-2-butyl)-hydrazine, No. 20.—Alkylidene compound (No. 19) (0.023 mole), 50 cc. ethanol, 0.1 Gm. catalyst, six and one-half hours for 577 cc. uptake (theory = 565); recrystallized twice from 50:50 benzene-ligroin (b. p. 60–68°).

N^1-Isonicotinyl-N^2-(2,6-dimethyl-4-heptyl)-hydrazine, No. 22.—Alkylidene compound (No. 21) (0.092 mole), 250 cc. ethanol, 0.8 Gm. catalyst, sixteen hours for 2,600 cc. uptake (theory = 2,400); distilled at 158° (10^{-3} mm.), then redistilled.

N^1-Isonicotinyl-N^2-(9-heptadecyl)-hydrazine, No. 24.—Alkylidene compound (No. 23) (0.023 mole), 150 cc. ethanol, 0.4 Gm. catalyst, two hours for 595 cc. uptake (theory = 600); distilled at 215–225° (2×10^{-3} mm., direct flame), redistilled at 195–210° (0.03 mm.), and again redistilled.

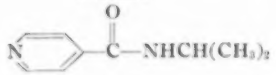
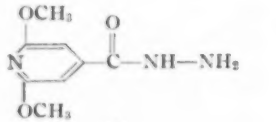
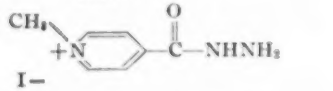
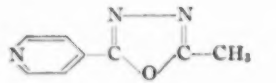
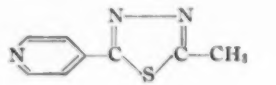
N^1-Isonicotinyl-N^2-(1-phenylethyl)-hydrazine, No. 27.—Alkylidene compound (No. 26) (0.017 mole), 50 cc. ethanol, 0.1 Gm. catalyst, six hours for 390 cc. uptake (theory = 415); recrystallized twice from 50:50 benzene-ligroin (b. p. 60–68°).

N^1-Isonicotinyl-N^2-(2-phenylpropyl)-hydrazine, No. 30.—Alkylidene compound (No 29) (0.089 mole), 125 cc. ethanol, 0.6 Gm. catalyst, five and one-half hours until a break in the hydrogenation curve at room temperature and 50 pounds per square inch gauge pressure of hydrogen; distilled at 120–200° (5×10^{-3} mm.), redistilled four more times.

N-Isopropyl Isonicotinamide, No. 31.—Ethyl isonicotinate (15.1 Gm., 0.10 mole) and isopropyl amine (17.7 Gm., 0.30 mole) were mixed in a glass-lined autoclave and heated for two hours at 120°. The cooled reaction mixture was stripped under vacuum on the steam bath and the residue was recrystallized twice from carbon tetrachloride to give 9.05 Gm. (55% yield) of product.

2,6-Dimethoxyisonicotinic Hydrazide, No. 32.—A mixture of 2,6-dichloroisonicotinic acid (9) (20.0 Gm., 0.104 mole) and a solution of sodium (8.0 Gm., 0.348 mole) in dry methanol (100 cc.) was heated for two hours at 130–140° in a glass-lined autoclave. The methanol was then evaporated and the residue was taken up in 300 cc. water, the solution was acidified and the precipitated product was recrystallized from methanol to give 11.2 Gm. (58% yield) of material, m. p. 222.5–224°; another

TABLE II.—MISCELLANEOUS ISONICOTINYL DERIVATIVES

No.	Structure	M. p., ° C.	Empirical Formula	Analyses					
				Calculated			Found		
				C	H	N	C	H	N
31.		108.5–110	$C_9H_{12}N_2O$	65.82	7.37	...	66.04	7.22	...
32.		170–171	$C_8H_{11}N_3O_3$	48.72	5.62	...	49.02	5.93	...
33.		210–213	$C_8H_7N_3O.CH_3I$	...	...	5.02	...	...	4.70
34.		150.5–151	$C_8H_7N_3O$	59.62	4.38	26.07	59.45	4.43	26.13
35.		155–155.5	$C_8H_7N_3S$[a]	54.22	3.98	...	54.39	4.07	...
36.		240.5–241.5	$C_{12}H_8N_4S$[b]	59.98	3.33	23.32	59.57	3.05	23.40

[a] Sulfur, Calcd.: 18.09; Found: 18.07.
[b] Sulfur, Calcd.: 13.34; Found: 13.19.

recrystallization gave pure 2,6-dimethoxyisonicotinic acid, m. p. 227–228°.

Anal.—Calcd. for $C_8H_9NO_4$: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.66; H, 5.06; N, 7.80.

The same acid was obtained when the methyl ester of 2,6-dichloroisonicotinic acid was methanolized. A mixture of methyl 2,6-dichloroisonicotinate (10) (13.0 Gm., 0.063 mole) and a solution of sodium (4.85 Gm., 0.21 mole) in dry methanol (45 cc.) was heated for two hours at 130–140° in a glass-lined autoclave. The cooled reaction mixture was rinsed from the autoclave with additional dry methanol, the precipitated sodium chloride was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in water, the solution was acidified with 4 *N* hydrochloric acid and the crude 2,6-dimethoxyisonicotinic acid (13.1 Gm.) was removed by filtration. After recrystallization from methanol it weighed 8.2 Gm. (67% yield) and melted at 226–227°.

2,6-Dimethoxyisonicotinic acid (5.0 Gm., 0.0273 mole) was heated on the steam bath for one-half hour with thionyl chloride (15 Gm., 0.126 mole); the excess thionyl chloride was removed by distillation, the last traces being purged by codistillation with 20 cc. dry toluene, to leave as the residue 2,6-dimethoxyisonicotinyl chloride hydrochloride which was used without further purification. To the crude acid chloride, there was added 50 cc. dry ethanol and after the mixture was warmed a few minutes the excess ethanol was evaporated to leave the crude ethyl 2,6-dimethoxyisonicotinate hydrochloride which was also used without further purification. The crude ester was heated on the steam bath for sixteen and one-half hours with 10 cc. of 100% hydrazine hydrate and 10 cc. of dry ethanol. The reaction mixture crystallized on cooling and recrystallization (charcoal) of the material from water gave 2.2 Gm. (41% yield) of pure hydrazide.

Isonicotinic Hydrazide Methiodide, No. 33.—The reaction of isonicotinic hydrazide with methyl iodide was less satisfactory than the following alternative procedure. A mixture of methyl isonicotinate (13.7 Gm., 0.10 mole), methyl iodide (16 Gm., 0.11 mole), and methanol (150 cc.) was refluxed for two hours and then cooled. Methyl isonicotinate methiodide crystallized and after recrystallization from ethanol it melted at 183.5–186° (dec.).

Anal.—Calcd. for $C_7H_7NO_2.CH_3I$: N, 5.02. Found: N, 4.70.

A mixture of methyl isonicotinate methiodide (5.0 Gm., 0.017 mole), hydrazine hydrate (1.2 Gm., 0.024 mole), and methanol (100 cc.) was warmed on the steam bath for fifteen minutes, then the crystalline precipitate formed was removed and recrystallized twice from 150-cc. portions of methanol. The purified material weighed 1.9 Gm.

2-Methyl-5-(4-pyridyl)-1,3,4-oxadiazole, No. 34.—A mixture of isonicotinic hydrazide (50.0 Gm., 0.365 mole) and acetic anhydride (44.7 Gm., 0.438 mole) was refluxed for four hours and then allowed to stand overnight. The partially crystalline reaction mixture was washed with three portions of dry ether, then 100 cc. of ethanol was added, and the mixture heated on the steam bath until homogeneous and filtered hot. Ligroin (50 cc., b. p. 60–68°) was added to the cooled filtrate and the resultant precipitate was removed by filtration and discarded. Additional ligroin was added to the now clear filtrate and, on standing, the solution deposited 21.5 Gm. (33% yield) of crystalline material m. p. 143–147°. After recrystallization from isopropanol the material weighed 12.1 Gm. and melted at 160.5–161.5°; another recrystallization from ethanol gave pure N^1-isonicotinyl-N^2-acetylhydrazine, m. p. 161–162°. The relative ineffectiveness of this material as an antitubercular substance was reported by Bernstein, *et al.* (7), but there is no record in the literature of its characterization.

Anal.—Calcd. for $C_8H_9N_3O_2$: C, 53.63; H, 5.06. Found: C, 53.93; H, 5.22.

The following procedure was patterned after that of Stolle and Hille (12) for a similar 1,3,4-oxadiazole. A mixture of N^1-isonicotinyl-N^2-acetylhydrazine (5.0 Gm., 28 mM) and acetic anhydride (9.0 Gm., 88 mM) was heated for seventeen hours at 225° in a pressure bottle. To the cooled reaction mixture there was added ether until no further precipitation occurred. The black precipitate was removed by filtration and discarded and the ethereal filtrate was extracted with water. The aqueous extract was made alkaline with sodium hydroxide and then evaporated on the steam bath, under vacuum, to a syrupy residue. This residue was leached with ethanol and the ethanol stripped from the leachings to leave a solid residue which was extracted with acetone. Concentration and chilling of the acetone extract gave 0.4 Gm. (9% yield) of the crystalline oxadiazole derivative which melted, after recrystallization from 3 cc. of ethanol, at 150.5–151°.

2-Methyl-5-(4-pyridyl)-1,3,4-thiadiazole, No. 35.—The following procedure was patterned after that used by Stolle and Johannissien (13) for a similar 1,3,4-thiadiazole. A mixture of N^1-isonicotinyl-N^2-acetylhydrazine (5.0 Gm., 0.0279 mole) and phosphorus pentasulfide (15.0 Gm., 0.0675 mole) was heated for seventeen hours at 250° (bath temperature). Three hundred cc. of 5% aqueous sodium hydroxide was added to the cooled reaction mixture and the insoluble material was removed by filtration and discarded. The alkaline filtrate was acidified with concentrated hydrochloric acid and another amount of dark brown insoluble material was removed by filtration and discarded. The now acidic filtrate was again made alkaline and was then extracted with three 100 cc.-portions of chloroform. Removal of the solvent from the combined dried chloroform extracts left the solid product which, after two recrystallizations (charcoal) from ethanol, weighed 0.5 Gm. (10% yield).

2,5-Di-(4-pyridyl)-1,3,4-thiadiazole, No. 36.—A solution of isonicotinonitrile (14) (10.0 Gm., 0.096 mole) in absolute alcohol (20 cc.) was mixed with a solution of 80 cc. of absolute alcohol saturated at room temperature with ammonia. This mixture was cooled in an ice bath and then saturated with hydrogen sulfide. Crystals began to separate after about five minutes and when the reaction mixture became nearly solid it was allowed to warm to and stand at room temperature for twenty-two hours. It was then chilled and filtered to give material which, after recrystallization from water, weighed

9.6 Gm. (72% yield) and melted at 209–210°. Another recrystallization from water gave pure thioisonicotinamide, m. p. 210°.

Anal.—Calcd. for $C_6H_6N_2S$: N, 20.27; S, 23.20. Found: N, 20.51 (Dumas), 20.18 (Kjeldahl); S, 23.00

A solution of thioisonicotinamide (5.7 Gm., 0.0412 mole) in 100% hydrazine hydrate (30 cc.) was heated for one hour at 130° (bath temperature) under a reflux condenser protected by a sodium hydroxide drying tube. Excess hydrazine hydrate was removed under vacuum and the remaining material was treated with excess 4 *N* hydrochloric acid. Insoluble material was removed by filtration and the filtrate was made alkaline with concentrated ammonium hydroxide. The resultant precipitate was recrystallized from *n*-propanol, then dissolved in dilute hydrochloric acid and reprecipitated by the dropwise addition of dilute sodium hydroxide solution. After another recrystallization of this material from *n*-propanol it weighed 1.1 Gm. (22% yield).

REFERENCES

(1) Shavel, J., Leonard, F., McMillan, F. H., and King, J. A., THIS JOURNAL, **42**, 402(1953).
(2) Selikoff, I. J., Robitzek, E. H., and Ornstein, G. G., *Quart. Bull. Sea View Hosp.*, **13**, 17(1952).
(3) Robitzek, E. H., Selikoff, I. J., and Ornstein, G. G. *ibid.*, **13**, 27(1952).
(4) Bosworth, D. M., Wright, H. A., and Fielding, J. W., *ibid.*, **13**, 52(1952).
(5) Fieser, L. F., in "Biological Antioxidants" (Transactions of the Third Conference), C. G. Mackenzie, Editor. Josiah Macy, Jr. Foundation, New York, N. Y. (1948), pp. 24–32.
(6) Shchukina, M. N., Pershin, G. N., Makeeva, O. O., Sazonova, E. D., Nikitskaya, E. S., Yanina, A. D., and Yakovleva, A. I., *Doklady Akad. Nauk S.S.S.R.*, **84**, 981 (1952).
(7) Bernstein, J., Lott, W. A., Steinberg, B. A., and Yale, H. L., *Am. Rev. Tuberc.*, **65**, 357(1952).
(8) Büchi, J., Labhart, P., and Ragaz, L., *Helv. Chim. Acta*, **30**, 507(1947).
(9) Bittner, K., *Ber.*, **35**, 2933(1902).
(10) Mayer, H., and von Beck, E. R., *Monatsh.*, **36**, 731 (1915).
(11) Mariella, R. P., and Leech, J. L., *J. Am. Chem. Soc.*, **71**, 3558(1949).
(12) Stolle, R., and Hille, H., *J. prakt. Chem.*, **69**, 481 (1904).
(13) Stolle, R., and Johannissien, A., *ibid.*, **69**, 474(1904).
(14) La Forge, F. B., *J. Am. Chem. Soc.*, **50**, 2477 1928).

Composition of Gum Turpentines of Pines

XVII. A Report on *Pinus montezumae* from Chiapas and *P. oocarpa* var. *trifoliata* and *P. durangensis* from Durango, Mexico*,†

By P. M. ILOFF, Jr., and N. T. Mirov‡

PINUS MONTEZUMAE FROM CHIAPAS

Pinus montezumae Lamb. is the name given an extremely variable group of pines which, for the lack of better knowledge, is commonly designated as a single species. Martinez (2) subdivides this species or rather the complex into six different species, four varieties, and five forms, namely, *P. montezumae* proper and its form—*macrocarpa* Martinez and variety *Lindleyi* Loudon; *P. durangensis* Martinez and its form *quinquefoliata* Martinez; *P. Hartwegii* Lindl., *P. rudis* Endl.; *P. Cooperi* Blanco and its variety *ornelasi* Martinez; *P. michoacana* Martinez and its varieties—*quevedoi*, *cornuta*, *cornuta* forma *nayaritana*, forma *procera*, and forma *tumida*, all named by Martinez.

Turpentine of *P. montezumae* from Michoacan has already been analyzed and the results reported in THIS JOURNAL (3). Great care was exercised to identify the trees correctly. Later, herbarium specimens were sent to Professor Maximino Martinez of the Institute de Biologia, Mexico, D. F., for verification. Turpentine from the Michoacan *Pinus montezumae* proved to consist of 98% of *d*-α-pinene.

Turpentine of presumably the same species from Chiapas, as reported in this paper, contained besides *d*-α-pinene appreciable amounts of limonene, *n*-heptane, and longifolene. These three ingredients we also found, although in much larger proportions, in the turpentine of *Pinus pseudostrobus* from Chiapas (1). The only explanation of this discrepancy that we can offer is that *Pinus montezumae* of the Chiapas locality (650 miles distant from the Michoacan area and separated by the lowlands of Tehuantepec) is a different pine, possibly a hybrid between *Pinus montezumae* and *P. pseudostrobus*. These two pines are very closely related, and hybridization is quite possible.

* Received January 14, 1953, from the Institute of Forest Genetics, California Forest and Range Experiment Station, U. S. Department of Agriculture, P. O. Box 245, Berkeley 1, Calif.
† Contributed by the California Forest and Range Experiment Station which is maintained by the Forest Service, U. S. Department of Agriculture in cooperation with the University of California, Berkeley. The work reported in this paper was aided through a grant from the Rockefeller Foundation.
‡ The oleoresin collecting trip was made by N. T. Mirov in the summer of 1950 with the assistance of the associates of Subtropical Geography, University of California. Thanks are due to Dr. Carl O. Sauer of the Department of Geography of the University for his interest in the project.

EXPERIMENTAL

Four liters of oleoresin were obtained from 20 mature trees at Rancho Nuevo, some 40 miles southeast of San Cristobal. The trees were photographed, and herbarium material was preserved for future references.

A batch of 3,000 Gm. of oleoresin was distilled under reduced pressure, so that at the end of the distillation, temperature had risen to 175°, and the pressure was reduced to 2 mm. Yield of turpentine was 25% of the trash-free oleoresin.

The turpentine possessed the following physical characteristics: d_4^{23}, 0.8441; n_D^{23}, 1.4612; $[\alpha]_D^{23}$, +19.2°. A batch of 550 Gm. of turpentine was fractionated by means of an 80-cm. Vigreux column with heated jacket. A reflux ratio of 1:10 was maintained. Results of the distillation are shown in Table I.

The low refractive index and density of fraction 1 indicated the presence of paraffin hydrocarbon. Therefore a portion of fraction 1 was shaken repeatedly with fresh portions of fuming sulfuric acid until it no longer became colored on shaking. Then the residual colorless liquid was washed with concd. sulfuric acid followed by aqueous sodium bicarbonate solution and then water. The hydrocarbon was dried over anhydrous sodium sulfate. The physical properties are compared with literature values below:

	n-Heptane from Fraction 1	*n*-Heptane[a]	
n_D	$1.3868^{22.5}$	1.38764^{20}	1.38517^{25}
d	0.6807_4^{23}	0.68368^{20}	0.67947^{25}
B. P.	97–98° at 760 mm.	98.428°	

[a] Rossini, *et al.*, "Selected Values of Properties of Hydrocarbons," Circular of the National Bureau of Standards C461, p. 39 (1947), U. S. Govt. Printing Office.

The infrared spectrum was identical with that of heptane for *P. pseudostrobus* Lindl. reported in a previous paper of this series (1). The yield of heptane was 88.5% of fraction 1. The low refractive index and boiling range of fraction 2 indicates heptane along with α-pinene. The turpentine of *P. montezumae* from Chiapas contains about 8.2% *n*-heptane.

Fractions 3 and 4 are mainly *d* and *dl*-α-pinene. α-Pinene was detected in fraction 4 by preparation of α-pinene nitrosochloride which, upon several recrystallizations from chloroform by addition of cold methanol, melted at 103–104°. No depression in melting point occurred upon admixture with the nitrosochloride prepared from α-pinene from *P. Cooperi*.

Judging from physical properties, fractions 5 and 6 contained α-pinene with small amounts of limonene. α-Pinene nitrosochloride was prepared from fraction 6. Oxidation of 20 ml. of fraction 6 with alkaline permanganate yielded no nopinic acid. When the densities and refractive indices of the fractions were plotted as ordinates and abscissae, respectively (5), there was no indication of β-pinene.

Fractions 7, 8, and 9 contain *l* and *dl*-limonene. Fraction 8 was redistilled over sodium in a 50-ml. Claisen flask to yield 3 fractions. The majority of the distillate was collected in the middle fraction which had the following properties: b^{760}, 176–177°; n_D^{23}, 1.4702; $[\alpha]_D^{23}$, −94.4°; d_4^{23}, 0.8396. A tetrabromide was prepared from the middle fraction. After two recrystallizations from methanol, the tetrabromide had a melting point of 103–103.5° and showed no depression upon admixture of authentic limonene tetrabromide.

Fraction 10 was a liquid with a pleasant sweet-smelling odor indicating the probable presence of oxygenated terpene derivatives. Upon further distillation a series of fractions were collected between 180° and 194° at atmospheric pressure. These fractions were evidently impure and their physical properties suggest a mixture of terpene esters and alcohols.

Judging by the physical properties of fractions 11, 12, and 13, they contain sesquiterpene. Fraction 13 was redistilled over sodium at reduced pressure. The bulk of the fraction distilled at 77–78° at 1.5 mm. of mercury and had the following physical properties: n_D^{23}, 1.5012; d_4^{24}, 0.9277; $[\alpha]_D^{24}$, +48.5°; M_D, 65.9. Three grams of the distillate were placed in 18 ml. of dry ether and kept in an ice salt bath while dry HCl gas was bubbled through for thirty minutes. The solution was then placed in the refrigerator for a week after which the ether was allowed to evaporate in an open dish. The residue was put in the refrigerator for another week and then the crystalline precipitate was removed and placed upon a clay plate. The crystals were recrystallized from glacial acetic acid yielding 1.2 Gm., m. p. 59°; mixed with longifolene dihydrochloride derived from turpentine of *P. cembroides*, m. p. 59°.

The turpentine of *P. montezumae* from Chiapas consists of 72% *d* and *dl*-α-pinene, 6–7% *l* and *dl*-limonene, 8% heptane, 8% longifolene and 1–2% oxygenated terpene derivatives.

PINUS OOCARPA VAR. TRIFOLIATA MARTINEZ

This pine differs from typical *Pinus oocarpa* Scheide by having three needles in each bundle instead of five. The three-needled variety occurs only in the mountains of the State of Durango. It was discovered by Cenobio E. Blanco and described by Martinez (2).

Pinus oocarpa and its four varieties occupy a large area, extending from southern Chihuahua, Mexico, to the mountains of Guatemala. Turpentine from *P. oocarpa typica* collected in central Mexico has already been analyzed and reported in THIS JOURNAL (3). It consisted almost entirely of a *d*-α-pinene. *P. oocarpa* from the State of Chiapas, southern Mexico, also has been investigated (1). Its turpentine was found to contain α-pinene, limonene, and a sesquiterpene longifolene. So it appears that *P. oocarpa* from central Mexico and *P. oocarpa* from southern Mexico (Chiapas) are biochemically different pines.

EXPERIMENTAL

The oleoresin of the variety *trifoliata* reported in this paper was collected under the supervision of C. E. Blanco near El Salto, Durango.

A batch of 2,700 Gm. of oleoresin was distilled under reduced pressure until all volatile ingredients were recovered. The temperature toward the end of the distillation reached 170°, and the pressure was reduced to 0.2 mm. Yield of turpentine was 23% of the trash-free oleoresin.

The physical characteristics of the gum turpentine of *P. oocarpa* var. *trifoliata* were as follows: d_4^{24}, 0.8588; n_D^{23}, 1.4682; $[\alpha]_D^{24}$, +39.8°.

A batch of 558 Gm. of the gum turpentine was distilled at reduced pressure through an 80-cm. heated-jacket Vigreux column. A 1:10 reflux ratio was maintained. When the temperature reached 88° at 10 mm., the pot residue was transferred to a smaller flask and the distillation was continued using a 3-inch Vigreux column. The results of the distillation are shown in Table I.

Fractions 1 and 2 are predominately α-pinene. The presence of α-pinene in fraction 1 was shown by the preparation of *dl*-α-pinene nitrosochloride which was converted to the nitrolpiperide. The nitrolpiperide was recrystallized twice from 95% ethanol and melted at 118–119°. A mixed melting point determination with *dl*-α-pinene nitrolpiperide derived from *P. echinata* turpentine showed no depression in melting point. *dl*-α-Pinene nitrosochloride, melting at 103.5–104° after two recrystallizations from chloroform by the addition of cold methanol, was prepared from fraction 2.

Fraction 3 was fractionated in a 12-inch glass helix-packed column. The boiling range at 760 mm. (155–170°) and other physical characteristics of the fraction obtained indicated that the fraction contained α-pinene and some higher boiling terpene, possibly Δ^4-carene. No nitrosate was formed in an attempt to identify Δ^3-carene in these fractions.

Fraction 5, redistilled over sodium at 760 mm. pressure, boiled at 180–185°, and its index of refraction, n_D^{23}, was 1.4799. A bromide was prepared by adding bromine to an alcohol-ether solution of fraction 5. After two recrystallizations from alcohol the bromide melted at 113–113.5°. On admixture with terpinolene tetrabromide the melting point was depressed below 108°.

Judging from its physical properties, fraction 6 is mostly sesquiterpene.

Fraction 7 was redistilled over sodium in a 3-inch Vigreux column. The higher-boiling fraction, amounting to 55% of the original fraction 7, had the following properties: n_D^{23}, 1.5115; d_4^{24}, 0.9508; $b^{2.5}$, 73–76°. One and one-half grams of the higher-boiling fraction was dissolved in 9 ml. of dry ether. Dry HCl gas was passed through the solution, which was kept between 0 and 5° by means of an ice-salt bath. After thirty minutes the solution was poured into a glass-stoppered bottle and left in a refrigerator one week. Then the ether solution was poured into a watch glass and the ether was allowed to evaporate. After a few days in the refrigerator, crystals formed; they were placed on a clay plate for a few hours and then recrystallized from glacial acetic acid and melted at 58–59°.

Fraction 8 was redistilled over sodium metal in a 3-inch Vigreux column. The higher-boiling fraction, amounting to 54% of the original fraction 8, had the following properties: n_D^{22}, 1.5023; $d_4^{22.5}$ 0.9327; b^3, 86–92°. Three grams of this higher-boiling fraction were treated with dry HCl gas in a manner similar to the sesquiterpene from fraction 7. A yield of 2.2 Gm. of crude crystals was obtained. Crystallized twice from glacial acetic acid, the crystals melted at 59° and showed no depression in melting point on admixture with longifolene hydrochloride from *P. pseudostrobus*.

The turpentine of *P. oocarpa* var. *trifoliata* is estimated to contain approximately 87% *d* and *dl*-α-pinene, about 5% unidentified terpenes, and 4.5% *d*-longifolene.

PINUS DURANGENSIS MARTINEZ

In 1938 Forest Engineer Cenobio E. Blanco described a majestic pine, "Un pino real" (2), growing in the State of Durango and possessing mostly 6 needles in a bundle. Subsequently, this pine was named by Pringle as *Pinus roseana* (4). Shaw included it first in *P. montezumae* and later classified it as *P. ponderosa*. Martinez (2) elevated it to the rank of species. One of us (N. T. Mirov), who observed this pine in the mountains of Durango, believes that Martinez was correct in calling it a separate species. Martinez also mentioned a form, *quinquefoliata*, of *Pinus durangensis* growing in Southern Chihuahua and having consistently five needles in a bundle. Durango pine resembles the Montezuma pine but its cones are comparatively small and its needles are more delicate. It also resembles *Pinus ponderosa*, but it lacks sharp prickles on the cone scales.

Turpentine from typical *Pinus montezumae* (from Michoacan) was shown to contain no other terpenes than *d*-α-pinene (3). On the other hand, all *Pinus ponderosa* varieties so far analyzed contained a great deal of Δ^3-carene. It was hoped that chemical analysis of turpentine of *Pinus durangensis* may cast some light on its relations to these two species.

EXPERIMENTAL

A batch of 2,200 Gm. of oleoresin of *P. durangensis* was distilled under reduced pressure. At the end of the distillation, temperature reached 180°, and pressure was reduced to 2 mm. Based on the trash-free weight of the oleoresin, 25% of turpentine was obtained.

The physical characteristics of the turpentine of *P. durangensis* were as follows: n_D^{21}, 1.4702; d_4^{22}, 0.8566; $[\alpha]_D^{22}$, +15.4°.

A batch of 498 Gm. of the turpentine was fractionally distilled through an 80 cm. jacketed and electrically heated Vigreux column equipped with a still head permitting about 1:10 reflux ratio. The results of the distillation are shown in Table I.

TABLE I.—FRACTIONAL DISTILLATION OF TURPENTINES OF *Pinus montezumae* FROM CHIAPAS, *Pinus oocarpa* VAR. *trifoliata*, AND *Pinus durangensis*

Fractions	Pressure, Mm. Hg	Boiling Range, ° C.	Distillate, %	Density	Index of Refraction, n_D^{23}	Specific Rotation, $[\alpha]_D^{23}$
Pinus montezumae from Chiapas (550 Gm. used) d_4^{23}						
1	755	97–120	8.9	...	...	...
2	755	120–154	1.7	...	1.4507	...
3	755	154–156.5	33.2	0.8490	1.4627	+36.4
4	755	156.5–159	22.0	0.8492	1.4644	+31.8
5	755	159–162	8.0	0.8488	1.4656	+19.3
6	755	162–166	7.0	0.8466	1.4670	− 1.1
7	10	55–60	4.0	0.8408	1.4707	−73.8
8	10	60–62	1.0	0.8378	1.4700	−92.0
9	10	62–65	0.6	...	1.4673	−85.1
10	10	65–85	1.6	...	1.4604	...
11	10	85–104	2.4	...	1.4962	...
12	2	85–90	0.7	...	1.4990	...
13	1.5	82–90	3.4	0.9059	1.5013	+48.0
Pot residue	...	...	1.6	...	...	...
Losses	...	...	3.3	...	...	...
Pinus oocarpa var. *trifoliata* (558 Gm. used) d_4^{23}						
1	10	44–47	78.4	0.8540	1.4652	+44.6
2	10	47–51	6.5	0.8531	1.4671	+34.2
3	10	51–56	5.5	0.8524	1.4693	+19.0
4	10	56–61	2.1	0.8499	1.4733	− 3.4
5	10	61–66	0.6	0.8548	1.4806	−11.7
6	10	66–88	0.4	0.8988	1.4965	+ 2.2
7	3	73–83	1.6	0.9469	1.5106	+20.8
8	3	83–93	2.6	0.9357	1.5034	+45.1
Pot residue	...	...	0.9	...	...	...
Losses	...	...	1.4	...	...	...
Pinus durangensis (498 Gm. used) d_4^{23}						
1	755	155–157	33.1	0.8558	1.4667	+28.8
2	755	157–158	24.3	...	1.4678	...
3	755	158–159	14.1	0.8566	1.4690	+18.9
4	755	159–160	5.6	...	1.4702	...
5	755	160–162	5.6	0.8577	1.4715	+ 6.7
6	755	162–164	4.4	0.8617	1.4727	− 1.4
7	755	164–166	2.1	0.8591	1.4742	−10.5
8	755	166–168	1.3	0.8577	1.4749	−14.1
9	755	168–172	1.6	0.8558	1.4751	−21.8
10	755	172–180	1.9	0.8518	1.4756	−24.6
11	15	60–85	0.6	0.8819	1.4817	...
12	15	85–95	1.1	0.9229	1.4905	...
13	15	95–105	1.0	0.9215	1.5001	+22.0
Pot residue	...	...	0.9	...	...	...
Losses	...	...	2.4	...	...	...

Fractions 1 to 6 possess the physical properties of mixtures of α and β-pinenes. Fraction 1 gave a good yield of *dl*-α-pinene nitrosochloride melting at 104°. From the nitrosochloride *dl*-α-pinene nitrosopiperidide was prepared; this melted at 118° after several crystallizations from ethanol. Judging from the physical properties, fraction 6 contained a high percentage of β-pinene. Upon oxidation with alkaline permanganate, sodium nopinate was produced. Nopinic acid, which had a melting point of 125.5–126° and gave no depression in melting point with authentic nopinic acid derived from *P. cooperi*, was formed from the sodium nopinate.

Fractions 7–10 contained an unidentified mixture of terpenes. Tests for Δ^3-carene yielded no nitrosate and tests for β-phellandrene yielded no nitrosite.

Fraction 11 was a mixture of terpenes and a sesquiterpene, and fraction 12 and 13 contained sesquiterpene. From fraction 13 a hydrochloride was prepared, which after two recrystallizations from glacial acetic acid melted at 120°. No depression was evident in a mixed melting point determination with authentic cadinene hydrochloride. The molecular refraction of fraction 13 was: M_D, 65.2.

Thus the turpentine of *P. durangensis* was estimated to contain approximately 71% *d* and *dl*-α-pinene, 19% *l*-β-pinene, 3% *d*-cadinene, and 7% unidentified material including pot residue and losses.

REFERENCES

(1) Iloff, P. M., Jr., and Mirov, N. T., THIS JOURNAL, **42**, 46(1953).
(2) Martinez, Maximino, "Los Pinos Mexicanos," 2nd ed., Ediciones Botas, Mexico, 1948.
(3) Mirov, N. T., THIS JOURNAL, **40**, 550(1951).
(4) Shaw, George Russell, "The genus *Pinus*," Pub. Arnold Arboretum No. 5, Cambridge, Mass., 1914.
(5) Sutherland, Maurice D., *Univ. Queensland Papers, Dept. Chem.*, **1**, 34(1948).

Chronic Oral Toxicity Studies of Triacetyl Diphenyl Isatin in Dogs and Rats*

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The results of acute and chronic toxicity studies of Triacetyl diphenyl isatin (TDI) on rats and on dogs are reported. The data obtained indicate TDI is only slightly toxic and cannot be classified as a high-grade irritant. In preliminary tests, human adult subjects showed increasing laxative effects with increasing doses. Relatively few undesirable side effects were reported, and those were moderate or slight in degree.

TRIACETYL DIPHENYL ISATIN (TDI) has been reported by Keimatsu and Yamaguchi (1) to be an effective laxative, superior in some respects to diacetoxy-diphenyl isatin (Endophenolphthalein). Based on the suggestion of Kaufmann (2) that the purgative action of hydroxyanthroquinone derivatives is to be ascribed to the di-(*p*-hydroxyphenyl)methane group, a series of compounds containing this grouping or modifications of this grouping was prepared and studied (3) with the purpose of finding a purgative agent sufficiently mild to be used in patients where any irritation is contraindicated. Recently the occurrence of a diphenyl isatin in prunes has been reported (4).

It has been suggested that acetyl groups may be split from TDI in the alkaline intestinal juices and that the effective cathartic action is the property of the insoluble diphenyl isatin (5). Diacetoxy-diphenyl isatin, however, has been quantitatively recovered from the feces when this material was given to rats in doses sufficient to produce a severe diarrhea (6).

Since no toxicity data were available on TDI, we have conducted the acute and chronic studies on rats and dogs presented herewith.

EXPERIMENTAL

Preparation of TDI.—Diphenyl isatin (prepared according to the method (7) of Baeyer and Lazarus) was refluxed for a few hours with acetic anhydride in the presence of fused sodium acetate, and poured into water; the TDI precipitated in an almost pure state. The compound was dissolved in chloroform, precipitated with ligroin and recrystallized from alcohol (8) (m. p. 201–202°).

Acute Toxicity; Rats.—Groups of adult, male and female, albino rats were given by stomach tube various doses of TDI dissolved in 2-ethylhexyl-phthalate (100 mg./cc.). Groups of 20 rats each were given doses in the range of 60 to 3,000 mg./Kg. Almost without exception rats in which a lethal effect was observed, died within twenty-four hours. The LD_{50} was calculated graphically to be 500 ± 96 mg./Kg. (9). Gross autopsy examinations on survivors two weeks later were negative.

Additional groups of rats (20 per group) were given intraperitoneally graded doses of TDI as a 50 mg./cc. solution in the same solvent. The dose range was 25 to 750 mg./Kg. The LD_{50} was calculated as 350 ± 45 mg./Kg. Gross autopsy examinations of surviving rats after two weeks revealed numerous superficial white flecks on the surfaces of the livers, kidneys, and spleens; the flecks were tentatively identified as unabsorbed TDI. A similar observation has been made in mice (6). In rats, by either route, TDI may be described as only slightly toxic.

Short-Term Feeding Studies; Rats.—In a preliminary study, groups of weanling female rats (10 per group) were maintained on diets containing O (control group), 0.05, 0.1, 0.2, and 1.0% TDI for a period of one month. The diets were prepared by mixing TDI into Purina Fox Chow meal. The diet and tap-water were provided *ad lib.* The 0.05% and control groups grew equally well. The 0.1% group failed to gain as rapidly for a week, but thereafter exhibited a normal growth rate. The other two groups showed severe interference with growth. Four of the 0.2% group and all of the 1.0% group died. The surviving rats were sacrificed after one month and organ weights were recorded. All values fell in normal ranges save an increase in the average liver weight for the 0.05% group (liver weights: control group 6.95 Gm.; 0.05% group 7.98 Gm.). At autopsy, samples of liver, kidney, pancreas, stomach, and small and large intestine were removed and prepared for histological examination. The customary hematoxylin and eosin stains were employed. There were no histological changes that were attributed to the ingestion of the TDI. Specifically there were no evidences of irritation in the gastrointestinal tract although all of the rats had shown some diarrhea, of severe degree in the 1.0% group.

Chronic Feeding Studies; Rats.—Weanling, male and female, albino rats (9 males and 9 females per group) were furnished diets containing the following percentages of TDI: 0 (control group), 0.05 0.075, 0.1, and 0.2%. The experiment was conducted in two parts; groups of 4 and groups of 5, respectively, were maintained separately (see Fig. 1). Initially the 0.05% groups grew about as well as did the control groups. Particularly in the case

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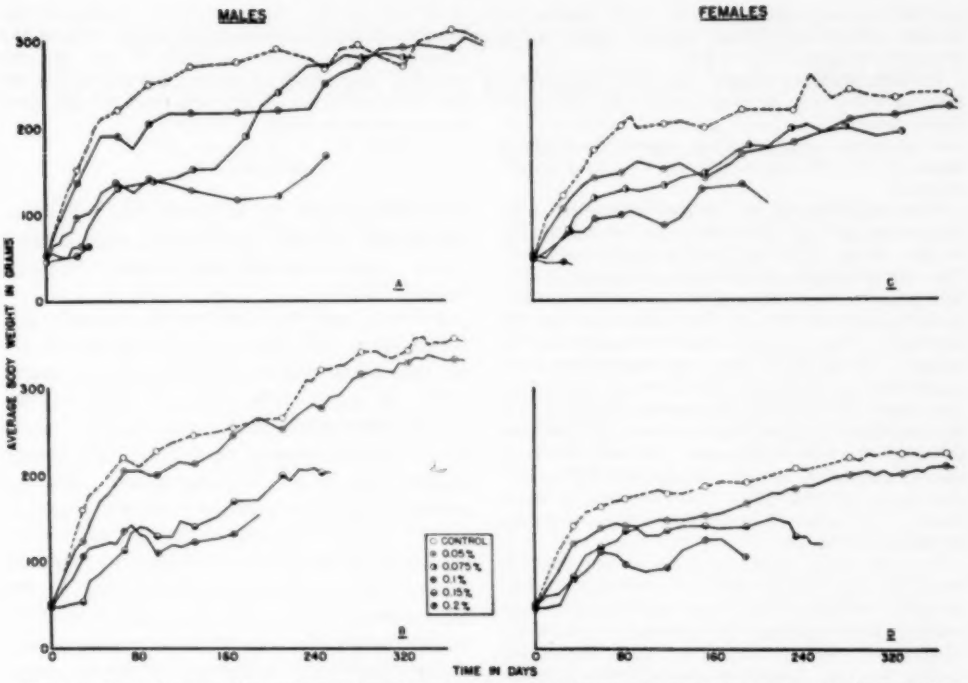

Fig. 1.—Growth curves for male and female rats fed TDI. The experiment is in two parts: in one, there were groups of five male (*A*) and five female (*C*) rats; in the other, groups of four male (*B*) and four female (*D*) rats. Diets contained the following percentages of TDI: ○—control groups; ⊙—0.05%; ◑—0.075%; ⊕—0.1%; ◒—0.15%; and ◓—0.2%. At the end of the year, the body weights of the control, 0.05 and 0.075% groups were practically identical.

of the female rats, however, some growth diminution appeared early in the test, although at the end of the year-long period there was no difference in the average body weights of the control groups and the 0.05% groups, male or female. With increasing percentages of TDI in the diets, growth retardation became more marked. The 0.1% groups grew hardly at all and the mortality was high. In the case of the 0.2% groups, the diarrhea was so severe that the percentage was reduced after a few days to 0.15%; even this level was not tolerated and most of the animals were dead in a month. In the 0.05 and 0.075% groups, the mortality was less than that of the control groups.

Blood samples were collected at the sixth, ninth, and twelfth months and examined for hemoglobin values, red blood cell counts, white blood cell counts, and differential counts. With the exception of a few aberrant values, the hemological data lay within normal ranges. Pooled urine collections were made from the control groups, the 0.05 and the 0.075% groups near the termination of the year period. On analysis normal sugar values were obtained but there was evidence of increasing amounts of protein with increasing doses of TDI.

At the end of the year period, the surviving rats were sacrificed by decapitation and gross autopsies were performed. Organ weights of heart, lung, spleen, liver, kidney, brain, stomach, and testes were recorded. All values were normal except those of the liver and testes; for these organs, weight increases were found with increasing percentages of TDI in the diets (Table I).

Sections of the following tissues were prepared for histological examination: lungs, stomach, small and large intestines, spleen, kidneys, heart, liver, gonads, and bone marrow. TDI should not be considered to be a high-grade irritant, because, despite the long period of diarrhea, the microscopic changes in the large bowel of rats given TDI were not markedly different from those seen in the control rats. The absence of specific changes in the livers

TABLE I.—AVERAGE LIVER AND TESTES WEIGHTS OF RATS AFTER ONE YEAR ON THE DIETS AS INDICATED

	Males			Females	
Diet	Average Body Wt., Gm.	Average Liver Wt., Mg./100 Gm. Body Wt.	Average Testes Wt., Mg./100 Gm. Body Wt.	Average Body Wt., Gm.	Average Liver Wt., Mg./100 Gm. Body Wt.
Control	329	30	6.8	223	31
0.05% TDI	311	34	7.3	215	32
0.075% TDI	282	42	9.9	194	44

and kidneys was worthy of note. No histological changes were observed that would account for the increased average testes weights.

Feeding Studies; Dogs.—In preliminary tests, dogs were found to develop a slight diarrhea when doses above 75 mg. were given. Lower doses did not interfere with normal bowel movements. Doses of 125–175 mg. produced a profuse watery diarrhea.

Three dogs were given for one year the following daily doses of TDI mixed in a diet of Purina Fox Chow: 50 mg., 100 mg., and 150 mg., respectively. The initial weight of each dog was about 10 Kg. Diarrhea was slight following the dose of 50 mg./day, more marked at 100 mg./day, and severe at 150 mg./day. Mucus in the stools increased with the dosage. Nevertheless, the dogs maintained body weight during the year period and in the case of the dog receiving 150 mg./day gained about 6 Kg. Hematological examinations were made at the start and near the end of the year; all values were in normal ranges, except an eosinophila (4%) in the dog given 150 mg./day. For each dog, a single urine analysis after 12 months gave negative values for sugar and protein.

The dogs were sacrificed at the end of the year by a large intravenous dose of barbiturate. Gross autopsy examinations were performed. Organ weights were normal. A thorough histological examination was made. The most interesting finding was the entirely normal appearance of the gastrointestinal tract in each dog. The absence of a highly irritating effect was clearly established. The liver and kidneys were normal.

Preliminary Tests on Human Subjects.—In a preliminary series of human tests, 20 male and 7 female, young adults (medical and graduate students) were selected on the basis of healthy gastrointestinal tracts. Careful gastrointestinal histories were taken and for a period of a week or more a record of the number of bowel movements was kept. None used cathartics, and no significant dietary variations were encountered. Only 1 subject took daily medication ($1^1/_2$ gr. thyroid daily). Fluid intake was recorded by each subject during the preliminary period; normal values were found.

The first testing procedure involved the administration of one 10 mg. tablet of TDI at night before retiring. The subjects were seen twelve to fourteen hours later and again after thirty-six to thirty-eight hours. The data reported included the time of the last bowel movement, the number of movements after taking TDI, the type of stools, and the incidence of side effects, e. g., cramping or urgency. After an interval of at least a week, a second dose, this time of 20 mg. of TDI, was administered and the routine was repeated. After another interval, a third dose of 30 mg. of TDI was given. At the conclusion of the test period, any subjective reactions to the series of tests were recorded.

Following the dose of 10 mg. of TDI about half of the subjects reported an increase in the number of bowel movements. In only 3 cases was there any noticeable softening of the stools. Following the 20 mg. dose (given to a total of 36 subjects) some cathartic effect was reported in about 80% of the cases. Approximately half of the individuals noted an increased softness of the stools. A dose of 30 mg. of TDI also produced an increase in the number of bowel movements in about 80% of the subjects. More than two-thirds of the subjects reported soft, semifluid watery stools. Relatively few undesirable side effects were reported and these were moderate or slight in degree.

SUMMARY

1. TDI given by stomach tube or intraperitoneally to adult rats is only slightly toxic (LD_{50}: orally, 500 mg./Kg.; intraperitoneally, 350 mg./Kg.).

2. In a year-long feeding study, male and female albino rats were maintained from weaning on diets containing the following percentages of TDI: 0, 0.05, 0.075, 0.1, and 0.2%. Little, if any, effect upon growth was observed in the 0.05% group; however, the 0.1 and 0.2% groups suffered severe growth retardation. Although mortality was high in the 0.1 and 0.2% groups, there were more survivors in the 0.05 and 0.075% groups than in the control groups. Periodic hematologic examinations gave values in the normal ranges. A single urine analysis near the end of the experiment showed increasing protein percentages with increasing dosages. Organ weights were normal except for increases in liver and testes weights with increasing percentage of TDI. Thorough histological examinations yielded evidences of irritation of the large bowel; considering the long period of diarrhea, however, the histological changes were not severe enough to classify TDI as a high-grade irritant. The tissues otherwise gave no evidence of toxic changes.

3. Three dogs fed 50, 100, and 150 mg. TDI, respectively, per day for one year exhibited a diarrhea of increasing severity with increasing dose. No marked changes were observed in the hemograms. Organ weights were normal. Thorough histological examinations revealed no evidence of specific toxic effects. The gastrointestinal tracts were normal throughout.

4. In preliminary tests, young adult human subjects showed increasing laxative effects with increasing doses, viz., 10, 20, and 30 mg. TDI.

REFERENCES

(1) Keimatsu, I., and Yamaguchi, E., *J. Pharm. Soc. Japan*, **55**, 1086(1935).
(2) Kaufmann, Z., *Angew. Chem.*, **40**, 831, 858(1927).
(3) Esson, A. P. T., Harrison, J., McSwiney, B. A., and Pyman, F. L., *Quart. J. Pharm. Pharmacol.*, **7**, 509(1934).
(4) Baum, H. M., Sanders, R. G., and Straub, G. J., THIS JOURNAL, **40**, 348(1951).
(5) *Pharm. Zig.*, **82**, 10(1937).
(6) Sanders, R. G., Baum, H. M., and Straub, G. J. THIS JOURNAL, **40**, 340(1951).
(7) Baeyer, A., and Lazarus, M. J., *Ber.*, **18**, 2641(1885).
(8) Lieberman, C., and Danaila, N., *ibid.*, **40**, 3592 (1907).
(9) Miller, L. C., and Tainter, M. L., *Proc. Soc. Exptl. Biol. Med.*, **57**, 261(1944).

A Morphological Study of the Seeds of *Pachyrrhizus erosus* (L.) Urban, the Yam Bean*,†

By BELLA Y. BAKER and MAYNARD W. QUIMBY‡

The morphology of the seeds of *Pachyrrhizus erosus* (L.) Urban has been described. Some information concerning the taxonomy of the genus is also included.

FOR MANY years *Pachyrrhizus*, commonly known as the yam bean, has been of economic importance to the natives of many of the tropical and subtropical countries. The tubers of the yam bean are sweet and succulent, and their high percentage of starch makes them a valuable source of food. The physiological activity and the therapeutic value of emulsions of the seeds for use as purgatives and of alcoholic tinctures for controlling body and head lice has long been known.

Current interest in this plant was stimulated during the second World War. It became necessary, because of the difficulty in obtaining raw materials, to explore various plants as possible sources of insecticides. As the value of the yam bean as a fish poison and pediculocide had long been established, it was natural to examine the bean as a possible insecticide.

Since no mention was found in the literature of any descriptive study of the seed, the authors undertook a morphological investigation. This study, including the determination of macroscopic and microscopic characteristics, showed several interesting features.

TAXONOMY

Pachyrrhizus, commonly known as the yam bean, is a genus of leguminous plants. It is believed to be indigenous to the Americas although it is now found growing both wild and cultivated in many parts of the tropical and subtropical sections of the world. De Candolle (1) founded the genus in 1825.

According to Altamirano and his associates (2), Hernandez commented on the desirable qualities of the tubers as early as 1615 and said that they were carried back to Spain buried in sand or as a sweet conserve. These authors (2) published an extensive report in 1907 on *Pachyrrhizus angulatus*, now believed to be *Pachyrrhizus erosus* (L.) Urban, giving a detailed account of the appearance of the plant, its physiological and therapeutic activity, as well as some chemical data. They mentioned that the tubers were frequently eaten raw as "fruit," roasted, or boiled in salted water. The report stated that infants, nursed by mothers who had eaten yam bean tubers, were seriously afflicted, and that some even died. Extracts of the seeds were recommended as parasiticides and antipsorics, and emulsions of the oil were used as cathartics.

Since that time perhaps the most important contribution, in so far as the taxonomy and botanical study of the yam bean is concerned, is the work done at Cornell University by Clausen (3). He made a complete study of the species and varieties of the yam bean in an effort to correlate the varied descriptions and the nomenclature of the species of *Pachyrrhizus*.

As the authors of this article have neither attempted to study the taxonomy nor have had adequate material available for studying the descriptive features of the plant, they have relied on Clausen's paper for descriptive information.

The spelling of *Pachyrrhizus* has varied throughout the years. De Candolle spelled it with a single "r," while in the *nomina conservanda* of the *International Rules of Nomenclature* (4) it was spelled with a double "r." In the *Index Kewensis* (5) it appears spelled with a single "r." For uniformity in this paper it is spelled throughout with a double "r."

MORPHOLOGY

Source and Preparation of Raw Materials.—The seeds for this study were certified for entry into the United States in April, 1945, by the Department of Agriculture of San Salvador, Central America. They were sent to Dr. Robert T. Clausen of Cornell University for study. He sent them, in turn, to the Massachusetts College of Pharmacy for further examination. A representative sample from the material worked upon has been deposited in the botanical collection of the Department of Biological Sciences, Massachusetts College of Pharmacy.

The dried seeds were macerated in warm water and appropriate sections for microscopic study were prepared in the usual ways.

Macromorphology.—The seeds of *Pachyrrhizus erosus* (L.) Urban exhibit many of the characteristics typical of leguminous seeds. Externally they are smooth, glossy, square to suborbicular, and laterally compressed seeds, varying from 5 to 8 mm. in length and from 5 to 7 mm. in width. The spermoderm is relatively hard and shows a sharp fracture. The latter characteristic may be due to the age of the material. The external color of the seeds varies from tan to reddish-brown to olive green. Each seed shows a dark elliptical indentation at the hilum.

A short, somewhat recurved funicle (Fig. 2, *1*), which is approximately 1 mm. high at one end and

* Received September 18, 1952, from the Massachusetts College of Pharmacy, Boston.

† This paper is based on a part of a thesis submitted by Bella Y. Baker to the Faculty of the Massachusetts College of Pharmacy in partial fulfillment of the requirements for the degree of Master of Science.

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0.25 mm. high at the other, remains attached to many of the seeds. When the funicle is removed a rim aril is observed, and this extends around the outer rim of the hilum (Fig. 2, *I*). This rim aril is elongated to form a flap on one side of the hilum. In some seeds this flap is located to the right and in some to the left of the hilum in relation to the micropyle. This flap always occurs on the inner face of the curved surface of the funicle, the curvature being dependent upon the location of the seed in the pod, whether it is attached to the right or to the left valve of the dehiscent fruit (legume).

This seed is campylotropous, the micropyle being located close to the hilum. The micropyle is readily visible under a hand lens. The raphe may also be seen in many seeds but in some cases it is obscured by the natural coloration of the spermoderm.

If the seeds are macerated in warm water for several days, the spermoderms can readily be slipped off thus exposing two firm, fleshy, cream colored cotyledons in each seed. These cotyledons are easily separated and appear rather homogenous except for scattered light patches. If the seeds are macerated for four or five days in warm water and the cotyledons then separated, definite signs of germination are visible.

Micromorphology.—Upon microscopic examination of the seed of *Pachyrrhizus erosus*, which will hereinafter be referred to as the yam bean, it is found divided into three definite regions, the spermoderm or seed coat, the scanty endosperm, and the embryo. The spermoderm (Fig. 1, *B*) is relatively thin, consisting of four definite cell layers and zones which are somewhat modified at the hilum end. These may be designated as (*a*) columnar cell layer, (*b*) hourglass cells, (*c*) outer spongy parenchyma, and (*d*) inner spongy parenchyma.

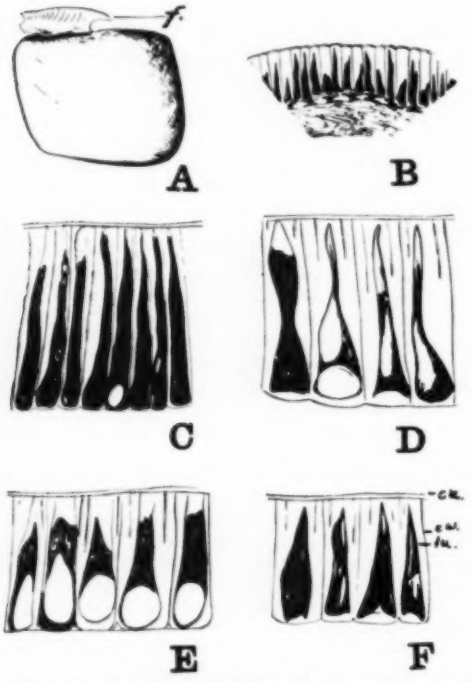

Fig. 1.—*A*.—Entire *Pachyrrhizus* seed, lateral view: *f*., funicle. *B*.—Seed coat in cross section. *C*, *D*, *E*, and *F*.—Columnar cells of seed coat: *cu*., cuticle; *cw*., cellwall; *lu*., lumen.

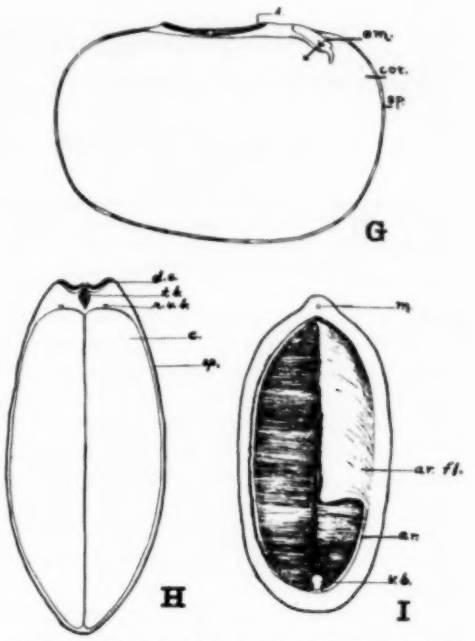

Fig. 2.—*G*.—Diagram of a median longitudinal section of the seed in plane of a cotyledon: *h*., hilum; *em*., embryo; *cot*., cotyledon; *sp*., seed coat. *H*.—Diagram of a transverse section of *Pachyrrhizus* seed: *d.c*., double row of columnar cells; *t.b*., tracheid island; *r.v.b*., recurrent vascular bundle; *c*., cotyledon; *sp*., seed coat. *I*.—Hilum area, enlarged: *m*., micropyle; *ar. fl*., aril flap; *ar*., rim aril; *v.b*., vascular bundle (trace).

The columnar cells (the *Malpighian cells* of Italian authors, also called "macrosclerids" and "osteosclerids") (6) occur as a single layer except in the hilum region. These cells are thick-walled, contiguous, and more or less hexagonal in transverse view. In lengthwise view they are much higher than broad.

Around the hilum slit two layers of columnar cells are found (Fig. 3, *G*), the outer layer being highly colored by a reddish-brown pigment.

The "light line," a colored band of different refractive power from the rest of the cell, which is very characteristic of columnar cells of many leguminous seeds, is not one of the outstanding features of the yam bean. In some hand-cut sections it is possible to observe a highly refractive area in the upper portion of the lower layer of columnar cells when the fine adjustment of the microscope is manipulated.

In surface sections the columnar cells appear much as a group of stone cells with deeply cut pore canals, usually seven or eight in number in each cell (Fig. 3, *J*). The outer ends of the columnar cells are

somewhat rounded and project into a mucilage layer, which separates the thin cuticle from the cells. In longitudinal view the walls of these cells appear unevenly thickened (Fig. 1, *C–F*). The lumen of each cell is rather broad at the base while relatively narrow at the upper end, thus giving it the appearance of a cone.

In spite of the hardness of the seed no lignification of the columnar cells was found when the material was warmed with phloroglucin-hydrochloric acid.

The ratio of the height to the width of the cells varies according to the location of the cells around the periphery of the seed. At the hilum end the cells are, on an average, about 75 μ high and 8 μ wide, a ratio of about 9:1. When viewed in sections cut in transverse plane the columnar cells midway down the flattened sides of the seeds average 42 μ in height and 12 μ in width, a ratio of about 3.5:1. At the base of the seed opposite the hilum in the same section, they exhibit a ratio of 4:1.

The density of the lumen content also varies. It appears extremely dense in cells near the hilum, while in cells at the center and base large vacuoles appear in the lumen. This condition may be due to hydration of the spermoderm prior to the preparation of the sections.

The subepidermal cells are commonly hourglass-shaped without evidence of any content (Fig. 3, *K*). Generally the cells are somewhat columnar but "waisted" and have expanded ends. Between these cells are wide intercellular spaces. At the hilum end several layers of such cells are seen, but here the cell outline becomes rather irregular with few definite intercellular spaces visible. The lower layers seem to merge into the spongy parenchyma, thereby losing all definite form.

The hourglass cells average about 45 μ in height and 25 μ in width. Many of these cells exhibit some lignification when treated with phloroglucin-hydrochloric acid.

Beneath these subepidermal cells are two regions of spongy parenchyma (Fig. 1, *B*). The outer region is of thin-walled parenchyma of irregular shape, while the inner region consists of very much compressed thin-walled spongy parenchyma. Distributed at intervals in the inner spongy parenchyma are small vascular traces which serve to nourish the seed. At the hilum end these regions become somewhat modified. The outer region beneath the hourglass cells consists of rather large thick-walled stellate cells, irregularly arranged, with numerous intercellular spaces. Immediately beneath the hilum there is an elongated "tracheid island" (Figs. 2, *H* and 3, *L*). This consists of a group of highly lignified cells exhibiting numerous simple pits. When viewed in a transverse section this tracheid island appears "tear-drop" shaped. It is believed that the tracheids serve to conduct nourishment to the developing ovule. Around the tracheid island and separating it from the stellate cells are several rows of thin-walled elongate cells without intercellular spaces.

Just within the spermoderm is a narrow region of rectangular endosperm cells. Near the hilum end this region is several cells in width but gradually becomes less extensive elsewhere.

The cotyledons make up by far the largest portion of the embryo. The remainder of the embryo exhibits a structure which is usual in this type of seed. Around the periphery of each cotyledon is a single layer of thick-walled epidermal cells (Fig. 3, *M*). On the inner face of each cotyledon the cells which will become the upper epidermis of the cotyledonary leaves on germination are tangentially elongate and average about 20–25 μ. The cells of the layers which face the spermoderm and which constitute the lower epidermises of the cotyledonary leaves are quadratic and average about 13 μ. Beneath the epidermis the reserve parenchyma cells (Fig. 3, *N*) of the mesophyll are thin-walled and they become progressively larger toward the center of the cotyledons. In section the cells exhibit many spherical bodies of fat and protein, as well as numerous small starch grains which vary from 3 to 9 μ in size. A few 2-,3-compound starch grains are found. Scattered here and there throughout the cotyledons are large glandular cavities lined by thin-walled parenchyma cells (Fig. 3, *O*).

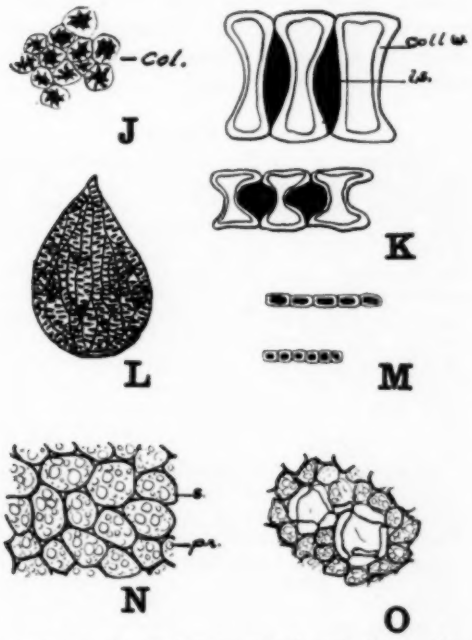

Fig. 3.—*J*.—Columnar cells in end view. *K*.—Hourglass cells: *cell w.*, cell wall; *i.s.*, intercellular space. *L*.—Tracheid island. *M*.—Epidermal cells of cotyledons. *N*.—Mesophyll cells of cotyledons: *s.*, starch; *pr.*, protein bodies. *O*.—Glandlike cavities of the cotyledonary mesophyll.

REFERENCES

(1) De Candolle, A. O., "Memoires sur la Famille de Légumineuses," Chez A. Belin, Imprimeur Libraire, Paris, 1825, p. 379.

(2) Altamirano, F., Ramirez, J., Villasenor, F. F., Armedaris, E., and Martinez del Campo, J., Datos para la Materia Médica Mexicana, *Inst. Méd. Nac.*, **4**, 1(1907).

(3) Clausen, R. T., "A Botanical Study of the Yam Beans (Pachyrrhizus)," N. Y. (Cornell) *Agr. Expt. Sta. Mem.*, 264(1944).

(4) "International Rules of Botanical Nomenclature," Science Press, Lancaster, Pa., 1935, p. 69.

(5) "Index Kewensis," vol. 2, ed. by Hooker and Jackson, Clarendon Press, Oxford, 1895, p. 399.

(6) Corner, E. J. H., "The Leguminous Seed," *Phytomorphology*, **1**, 118(1951).

The Synthesis of Fluoro-bromo Derivatives of Benzoic Acid to be Evaluated as Radiographic Opaques*

By CHARLES H. SPRAGUE,† GUSTAV E. CWALINA, and GLENN L. JENKINS

Seven new compounds: 3,5-dibromo-4-fluorobenzoic acid and its methyl, ethyl, *n*-propyl, and isopropyl esters, 3,5-dibromo-4-fluorohippuric acid, and 3,5-dibromo-4-fluorobenzamide have been synthesized. Three new compounds which were intermediates have also been made. They are: 3,5-dibromo-4-toluenediazonium fluoborate, 3,5-dibromo-4-fluorotoluene and 3,5-dibromo-4-fluorobenzoyl chloride. The opaque properties of 3,5-dibromo-4-fluorobenzoic acid, 3,5-dibromo-4-fluorohippuric acid, and ethyl 3,5-dibromo-4-fluorobenzoate were equal or superior to those of tetraiodophenolphthalein when radiographed in equivalent amounts in single tests on mice and indicate that the compounds are worthy of further study.

SOON AFTER the discovery of X-rays by Roentgen their application to medical diagnosis was begun. Contrast media, agents placed within the body to cause a certain area of that body to appear on an X-ray film, have been developed from relatively crude materials such as metal sounds and bags of 'ead solutions (1) to the selective agents currently being used. What was said to be the greatest advance in the field of radiographic opaques was the introduction by Graham, Cole, and Copher (2), in 1924, of tetrabromophenolphthalein as a diagnostic aid. Here was an agent which when taken by mouth would concentrate in the gallbladder in sufficient quantities to allow it to be visualized by X-rays.

Most radiographic opaques used today are iodinated compounds. In many cases they give satisfactory radiographs but it is well known that their use is too often attended by untoward side reactions. These include lacrimation, salivation, coughing spells, nausea, vomiting, fall in blood pressure, flushing of the face, choking sensation, and cyanosis. Although these symptoms usually disappear after a time, fatalities have occurred (3).

It has been said that the opacity of an element is proportional to the cube of its atomic weight (4). This is probably a misinterpretation of Hull's law which shows that the coefficient of absorption of all elements for X-rays varies approximately as the cube of the atomic number except in the immediate vicinity of one of the characteristic wave lengths of an element (5). It has been shown by Binz, *et al.* (6), and Hull's law affirms it, that bromine has better opaque properties than iodine.

It has been reported that fluorine stabilizes other halogens in aliphatic compounds when the two are on adjacent carbon atoms (7). This led to the supposition that fluorine has the same effect when attached to an aromatic ring. Verification of this supposition seems to be offered by the work of Mittelstaedt and Jenkins (8). Results of toxicity tests of some fluoro-iodo compounds which they prepared showed a low order of toxicity. These facts led to the belief that a more satisfactory radiographic opaque might be discovered through the synthesis of certain bromo-fluoro compounds.

EXPERIMENTAL

Preparation of 3,5-Dibromo-4-toluenediazonium Fluoborate.—An adaptation of the method of Schiemann and Winkelmuller (9) for the preparation of aromatic fluorine compounds was used. 4-Amino-3,5-dibromotoluene, prepared according to Hickinbottom (10), 255 Gm. (1.0 mole), was diazotized at 0–5° in 525 Gm. (2.5 moles) of fluoboric acid, 42%, with 69 Gm. (1.0 mole) of sodium nitrite. The diazonium fluoborate was collected on a Büchner funnel, washed with successive portions of cold distilled water, methyl alcohol and ether and air-dried for twenty-four hours. The yield was 350 Gm. (97.5%), decomposition temperature, 195–200°.

Preparation of 3,5-Dibromo-4-fluorotoluene.—The 3,5-dibromo-4-toluenediazonium fluoborate was thermally decomposed in a suitable apparatus (9). The crude 3,5-dibromo-4-fluorotoluene was washed out of the apparatus with ether. After filtering the ether solution and drying it over anhydrous sodium sulfate, the ether was removed by distillation and the residue vacuum distilled. The yield was 70 Gm. (28.1%), b. 118–125° at 21 mm. pressure.

Preparation of 3,5-Dibromo-4-fluorobenzoic Acid.—The oxidation was carried out by a general procedure (11). The 3,5-dibromo-4-fluorotoluene, 70 Gm. (0.28 mole), was oxidized in 500 cc. of acetic acid with 39 Gm. (0.39 mole) of chromic anhydride.

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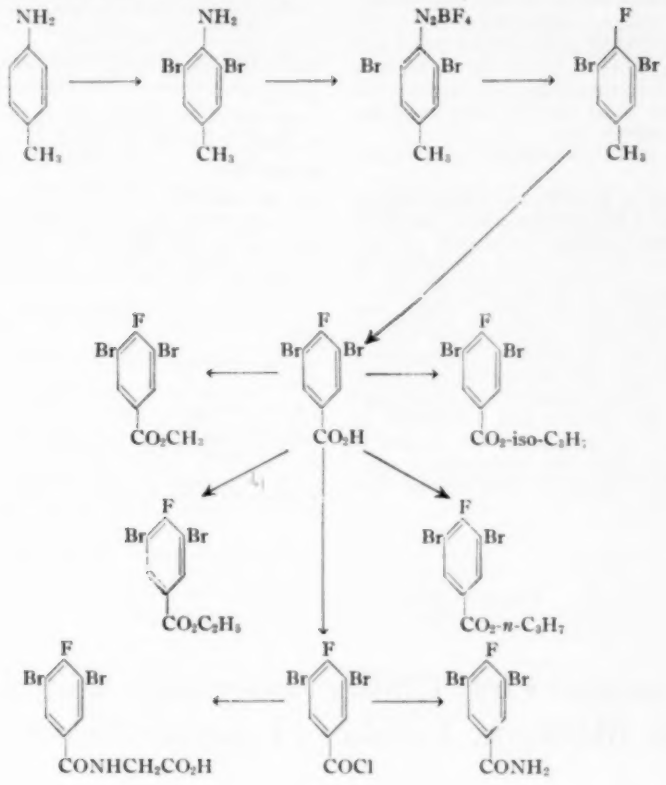
OUTLINE OF LABORATORY WORK



The crude acid was precipitated by the addition of distilled water and collected on a Büchner funnel. It was purified by dissolving it in ammonia solution, digesting with activated charcoal, filtering and precipitating it again with hydrochloric acid. The yield was 28.6 Gm. (36.8%), m. 234.5–235°.

Anal.—Calcd. for $C_7H_3O_2Br_2F$: neutr. equiv., 297.91. Found: neutr. equiv., 296.3.

Preparation of Esters of 3,5-Dibromo-4-fluorobenzoic Acid.—A modification of an esterification process described in Adkins and McElvain (12) was used. The 3,5-dibromo-4-fluorobenzoic acid, 2.97 Gm. (0.01 mole), was refluxed in 20 cc. of the appropriate alcohol saturated with hydrogen chloride. The esters were recrystallized from ethyl alcohol. Methyl ester: yield, 28%, m. 88–88.5°; ethyl ester: yield, 55%, m. 80–80.5°; *n*-propyl ester: yield, 12%, m. 34–35°; isopropyl ester: yield, 21%, m. 56–57°.

Preparation of 3,5-Dibromo-4-fluorobenzoyl Chloride.—An adaption of the method described in Gattermann and Wieland (13) was employed. The 3,5-dibromo-4-fluorobenzoic acid, 14.9 Gm. (0.05 mole), was refluxed with 17.8 Gm. (0.15 mole) of thionyl chloride in 50 cc. of dry benzene for sixteen hours. The excess thionyl chloride was removed by distillation and the residue dissolved in dry benzene for use in the following experiments.

Preparation of 3,5-Dibromo-4-fluorohippuric Acid.—The 3,5-dibromo-4-fluorohippuric acid was prepared according to the method which Ingersoll and Babcock (14) used to prepare hippuric acid. One-half of the benzene solution of 3,5-dibromo-4-fluorobenzoyl chloride prepared above and a solution of 4 Gm. (0.1 mole) of sodium hydroxide in 10 cc. of distilled water were added simultaneously to a stirred solution of 7.5 Gm. (0.1 mole) of glycine in 75 cc. of distilled water. The additions were made at such a rate that the reaction mixture was always slightly alkaline. The crude acid was precipitated by acidification and collected on a Büchner funnel. It was digested in carbon tetrachloride in which it is insoluble and the carbon tetrachloride removed by filtration. The 3,5-dibromo-4-fluorohippuric acid was recrystallized from boiling distilled water. The yield was 2.0 Gm., m. 210–211°.

Anal.—Calcd. for $C_9H_6O_3NBr_2F$: N, 3.95. Found: N, 3.87.

Preparation of 3,5-Dibromo-4-fluorobenzamide.—The 3,5-dibromo-4-fluorobenzamide was prepared according to the method described in Gattermann and Wieland (15) for the preparation of benzamide. The remainder of the benzene solution of 3,5-dibromo-4-fluorobenzoyl chloride prepared above was mixed with 20 Gm. of powdered ammonium

carbonate in an evaporating dish and heated with stirring until the odor of the 3,5-dibromo-4-fluorobenzoyl chloride was no longer discernible. The mixture was then stirred with distilled water and the undissolved portion collected on a Büchner funnel. The 3,5-dibromo-4-fluorobenzamide was recrystallized from ethyl alcohol. The yield was 1.5 Gm., m. 207.5–208°.

Anal.—Calcd. for $C_7H_4ONBr_2F$: N, 4.717. Found: N, 4.72.

Test for Opacity of 3,5-Dibromo-4-fluorobenzoic Acid, 3,5-Dibromo-4-fluorohippuric Acid and Ethyl 3,5-Dibromo-4-fluorobenzoate.—Solutions or suspensions of these in peanut oil were compared with the opaque properties of an aqueous solution of tetraiodophenolphthalein. The latter solution was made to contain 100 mg. of iodine per cubic centimeter and the others contained an equimolecular quantity of bromine. One-half cubic centimeter portions of the opaque solutions or suspensions were placed in the stomach and peritoneum of anesthetized mice and radiographed. Results of the tests revealed that the bromo-fluoro compounds gave better radiographs of the stomach than the iodo compound.

REFERENCES

(1) Greene, A. B., *X-Ray Technician*, **9**, 231 (1938).
(2) Graham, E. A., Cole, W. H., and Copher, G. H., *J. Am. Med. Assoc.*, **84**, 14(1925).
(3) "New and Non-Official Remedies—1951," American Medical Association, Chicago, Ill., 1951, pp. 280–281.
(4) Greenbaum, F. R., and Peters, A. F., *Radiology*, **35**, 45(1940).
(5) Hull, A. W., and Rice, M., *Phys. Rev.*, **8**, 326(1916).
(6) Binz, A., Rath, C., Maier-Bode, H., and Herman, K., *Angew. Chem.*, **45**, 713(1932).
(7) Henne, A. L., and Midgley, T., *J. Am. Chem. Soc.*, **58**, 882(1936).
(8) Mittelstaedt, S. G., and Jenkins, G. L., THIS JOURNAL, **39**, 4(1950).
(9) Schiemann, G., and Winkelmuller, W., in Blatt, A. H., "Organic Syntheses," Coll. Vol 2, John Wiley & Sons, New York, 1943, p. 299.
(10) Hickinbottom, W. J., "Reactions of Organic Compounds," 2nd ed., Longmans, Green and Co., New York, 1948, p. 323.
(11) Cason, J., and Rapoport, H., "Laboratory Text in Organic Chemistry," Prentice-Hall, New York, 1950, p. 170.
(12) Adkins, H., and McElvain, S. M., "Practice of Organic Chemistry," 1st ed., McGraw-Hill, New York, 1925, p. 221.
(13) Gattermann, L., and Wieland, H., "Laboratory Methods of Organic Chemistry," 22nd ed., Macmillan and Co., London, 1932, p. 110.
(14) Ingersoll, A. W., and Babcock, S. H., in Blatt, A. H., "Organic Syntheses," Coll. Vol. 2, John Wiley & Sons, New York, 1943, p. 328.
(15) Gattermann, L., and Wieland, H., "Laboratory Methods of Organic Chemistry," 22nd ed., Macmillan and Co., London, 1932, p. 119.

Experimental Oral Cholecystography with a New Contrast Medium, Teridax (Triiodoethionic Acid)*

By S. MARGOLIN, I. R. STEPHENS, M. T. SPOERLEIN, A. MAKOVSKY, and G. B. BELLOFF

Teridax (triiodoethionic acid), a new, oral cholecystographic medium was evaluated in non-anesthetized, trained dogs. At 100 mg./Kg., Teridax visualized the gall bladder more distinctly than 300 mg./Kg. iodoalphionic acid. The new compound at 150 mg./Kg. was as effective as 300 mg./Kg. of iodopanoic acid. Maximal opacity occurred between eight and ten hours with both Teridax and iodoalphionic acid. The safety of Teridax is indicated by acute toxicity data obtained in mice, rats, guinea pigs, and dogs. Blood determinations showed normal total and differential white blood cell counts, red blood cell counts, blood sugars, and hemoglobin values in all animals. Gross and microscopic examination showed no pathological changes in any organs examined. Liver and kidney function tests in dogs chronically medicated with Teridax were normal. No emetic reactions occurred in dogs, nor was there any evidence of gastric irritation. Pharmacodynamic tests with anesthetized dogs have not shown any deleterious effects upon respiration or the cardiovascular system. Clinical data indicate a close parallel between the gall-bladder opacity and definition, absence of colon opacity or gastrointestinal irritation, and the safety of Teridax in laboratory animals and in man.

ORAL CHOLECYSTOGRAPHIC agents have attained wide clinical acceptance in the routine diagnosis of gall-bladder disease. For the past decade, Priodax (iodoalphionic acid), has been the standard cholecystographic contrast medium. Compared to tetraiodophenolphthalein, iodoalphionic acid produces fewer undesirable side effects, and serious toxicity is rare. Nevertheless, the occurrence of these side actions continued to be objectionable and prompted continued synthetic programs directed toward more satisfactory substances. In our laboratories, experimental studies (1) were directed toward finding a compound which would provide: (*a*) freedom from nausea, diarrhea, dysuria, emesis, irritation of mucous membranes; low systemic toxicity; (*b*) clearly defined shadow, but one which would not be so dense as to obscure

* Received January 28, 1953, from the Pharmacology Laboratories, Schering Corp., Bloomfield, N. J.

the presence of stones; (*c*) visualization of the gall bladder without causing interfering shadows in the colon.

Preliminary laboratory findings with Teridax [alpha-ethyl beta - (2,4,6, - triiodo - 3 - hydroxy phenyl) propionic acid], suggested that this compound might possess the properties outlined above. In further experimental comparisons, Teridax produced gall-bladder shadows superior to those obtainable with either iodoalphionic acid, or Telepaque (iodopanoic acid), as evidenced by roentgenogram density and definition.

Outstanding was the absence of emesis or retching with Teridax in the several dogs tested, whereas iodoalphionic acid and iodopanoic acid elicited these undesirable reactions in the same dogs. Teridax has low systemic toxicity, and a therapeutic index in animals comparable to that of iodoalphionic acid.

EXPERIMENTAL

Oral Cholecystography in Dogs.—Experimental evidence suggests that the dog is the most suitable laboratory animal for the study of new cholecystographic compounds (3–5). Dogs weighing 7–11 Kg. were kept in the laboratory over several months, so that the same animals could be employed in a comparative series of tests with the various dosages of contrast media. In the experiments reported below, each of five dogs received comparable dosage regimens of the three substances used (Table I). The animals were trained to maintain their position under the roentgen tube; no anesthetic was required. The animals were fasted during the experiments except when visualization persisted for more than twenty-four hours. On such occasions, dogs were fed one pint of low-fat milk at twenty-eight hours after the oral administration of the compound, but were not fed solid food until after the forty-eight-hour picture. For all dogs, at least two weeks elapsed between each cholecystographic series.

The compounds were given orally in gelatin capsules. Roentgenograms were taken hourly for ten hours, after the administration of the contrast agent. Additional exposures were made at twenty-four hours, and at irregular intervals thereafter until a gall-bladder shadow no longer appeared. An Animagraph X-ray unit was employed with instrument settings at 80–88 KVP and 15 ma., and exposures of one-third second. Films were processed under standard conditions. Scorings of cholecystograms according to the method of Hoppe (2) were made by two or three observers to evaluate gall-bladder opacity and definition:

CHOLECYSTROGRAPHIC INDEX

4 *Excellent:* A sharp outline of the gall bladder with sufficient intensity to make the gall bladder stand out in brilliant contrast with the surrounding tissues.

3 *Good:* A distinct shadow of the gall bladder with satisfactory intensity and good definition.

2 *Fair:* A faint shadow of the gall bladder with faint, but visible definition.

1 *Poor:* Faint evidence of gall-bladder concentration as shown by an area of increased density in the region of the gall bladder, but with no definition.

0 *Failure:* No visualization whatsoever.

The following information was recorded: (*a*) hourly scores of gall-bladder visualization, (*b*) earliest maximal visualization, (*c*) presence of contrast agent in the large bowel, (*d*) occurrence of retching and/or vomiting.

The average maximal visualization obtained with the various compounds is summarized in Table I. Teridax at 150 mg./Kg. produced an opacity and definition equal to that obtained with 300 mg./Kg. of iodopanoic acid. At 100 mg./Kg., Teridax was distinctly superior to 300 mg./Kg. of iodoalphionic acid.

TABLE I.—ORAL CHOLECYSTOGRAPHIC STUDIES IN DOGS

Compound	Dosage, Mg./Kg.	Number of Dogs	Average Cholecystographic Index
Triiodoethionic acid (Teridax)	300	5	3.2
	150	5	3.6
	100	3	3.5
	75	2	2.0
Iodoalphionic acid	300	5	2.1
	150	5	1.1
Iodopanoic acid	300	5	3.1
	150	4	2.3

The maximal gall-bladder opacity and definition with Teridax and iodoalphionic acid occurred at eight to ten hours. However, iodopanoic acid attained maximal visualization at approximately seven hours.

To a varying degree, the large bowel was visualized by these agents. Colon shadows were least with Teridax and iodoalphionic acid; greatest with iodopanoic acid. At 300 mg./Kg., iodoalphionic acid appeared in the colon *after* the maximal visualization in three of five dogs, at the same time in one, and *before* in the fifth. In two of three dogs studied, Teridax at 300 mg./Kg. appeared in the colon *after* the gall bladder was visualized to a maximal degree; in the third dog, the contrast medium appeared concomitantly in the colon and the gall bladder. At 150 mg./Kg., Teridax, in two of four dogs, appeared in the colon after the earliest maximal visualization of the gall bladder; in the third dog, at the same time. In the fourth dog, a dense spot (apparently an unabsorbed tablet fragment) was noted in the colon just prior to visualization of the gall bladder. With iodopanoic acid at 300 mg./Kg., opacity of the colon occurred *before* maximal visualization of the gall bladder in two of four dogs; at the same time in the remaining two. In no instance did Teridax or iodoalphionic acid opacify the large bowel *before* maximal visualization of the gall bladder.

Acute Toxicity in Rodents.—The acute toxicity for Teridax was determined in Manor mice, Sprague-Dawley rats, and Manor guinea pigs. The median

TABLE II.—ACUTE TOXICITY OF TERIDAX IN RODENTS

Substance	Species	Mode of Administration	Number of Animals	LD_{50} and Confidence Limits,[a] Gm./Kg.
Triiodoethionic	Mouse	Oral	47	1.850 (1.640 to 2.090)
		Intraperitoneal	60	0.440 (0.400 to 0.485)
	Rat	Oral	18	Greater than 5.0
		Intraperitoneal	27	0.648 (0.528 to 0.794)
	Guinea pig	Intraperitoneal	29	0.570 (0.525 to 0.619)
Iodoalphionic	Mouse	Oral	42	2.950 (2.618 to 3.320)
		Intraperitoneal	64	0.640 (0.576 to 0.710)
	Rat	Intraperitoneal	50	0.510 (0.430 to 0.605)
	Guinea pig	Intraperitoneal	42	0.930 (0.820 to 1.065)

[a] $P = 0.05$.

lethal dose (LD_{50}) and confidence limits, computed by the method of Litchfield and Wilcoxon (6), are summarized in Table II. In toxic doses, Teridax or iodoalphionic acid caused sedation, generalized central nervous system depression, followed by intermittent colonic convulsions and dyspnea. The immediate cause of death was respiratory failure.

Intravenous Infusion Toxicity in Dogs.—Six mongrel dogs weighing 6.0 to 15.8 Kg. were anesthetized with morphine sulfate subcutaneously, plus sodium pentobarbital intravenously (Table III). An additional four dogs weighing 9.0 to 12.0 Kg. were anesthetized with chloral hydrate, intravenously. Blood pressure was recorded manometrically by cannulation of the right carotid artery. The compound was dissolved in water and dilute NaOH, brought to *p*H 7.5 with dilute HCl, and infused into the right femoral vein. Pulse and respiration rates were taken at intervals throughout the experiment.

The average intravenous lethal dose for Teridax in dogs anesthetized with morphine-sodium pentobarbital was 481.7 mg./Kg.; and in dogs anesthetized with chloral hydrate, 203.4 mg./Kg. Teridax was significantly more toxic to dogs anesthetized with chloral hydrate than to those anesthetized with morphine plus sodium pentobarbital. Teridax produced no significant change in blood pressure or respiration until approximately one-half of the infusion was given. Thereafter, a gradual decline in respiration and pulse rates occurred (Table IV).

TABLE III.—INTRAVENOUS TOXICITY OF TERIDAX IN DOGS

Dog Number	Body Weight Kg.	Anesthetic	Dose, Mg./Kg.	Mode	Solution, %	Rate of Infusion: Cc./Min.	Rate of Infusion: Mg./Kg./Min.	Lethal Dose, Mg./Kg.
1,867	10.0	Morphine sulfate	5.2	sc	1.0	1.35	1.36	506
		Sodium pentobarbital	24.0	iv				
1,874	6.0	Morphine sulfate	5.3	sc	0.6	0.94	0.93	443
		Sodium pentobarbital	11.0	iv				
1,950	8.2	Morphine sulfate	5.1	sc	1.0	0.84	1.0	303
		Sodium pentobarbital	27.8	iv				
1,965	9.5	Morphine sulfate	5.0	sc	1.0	1.45	1.5	602
		Sodium pentobarbital	22.0	iv				
1,991	15.8	Morphine sulfate	2.5	sc	1.0	1.8	1.1	347
		Sodium pentobarbital	18.2	iv				
2,011	14.5	Morphine sulfate	5.0	sc	1.2	2.6	2.1	689
		Sodium pentobarbital	16.5	iv				
1,873	9.0	Chloral hydrate	277.0	iv	1.0	2.3	2.6	265
1,993	9.3	Chloral hydrate	258.0	iv	1.0	0.4	0.4	60
1,996	12.0	Chloral hydrate	213.0	iv	1.0	0.4	0.3	103
2,004	10.9	Chloral hydrate	264.0	iv	1.2	1.2	1.2	385

TABLE IV.—EFFECT OF TERIDAX ON PULSE AND RESPIRATION RATES IN DOGS

Dog Number	Pulse Rate/Respiration Rate (Per Minute): Control	20 Minutes After Onset of Infusion	Middle of Infusion	Just Prior to Death
	Morphine-Sodium Pentobarbital Anesthesia			
1,867	120/10	100/8	84/4	54/1
1,874	80/6	66/6	90/4	72/2
1,950	160/4	158/4	138/6	100/4
1,965	98/12	94/8	84/6	68/2
1,991	118/8	74/7	90/6	92/2
2,011	112/23	106/21	100/12	82/5
	Chloral Hydrate Anesthesia			
1,873	152/35	130/38	110/36	56/2
1,993	156/58	134/60	130/70	104/32
1,996	118/24	126/27	124/38	96/0
2,004	120/21	124/22	76/8	88/2

TABLE V.—AVERAGE BLOOD VALUES FOR MICE FOLLOWING REPEATED DOSES OF TERIDAX

Teridax Dosage, Mg./Kg./Day	No. of Animals	Red Blood Cell Counts Per Cu. Mm. Million	White Blood Cell Counts Per Cu. Mm.	Hemoglobin, Gm./100 cc.	Differential Count				
					Neutrophils, %	Lymphocytes, %	Monocytes, %	Basophils, %	Eosinophils, %
900	7	11.1	15,757	14.1	32.5	66.1	1.1	0.2	0.1
600	10	10.3	16,340	14.0	32.7	66.0	1.1	0.1	0.1
300	10	12.6	15,038	14.3	31.1	68.1	0.5	0.2	0.1
Controls	10	10.6	13,035	14.1	31.9	66.6	1.2	0.3	0.2

TABLE VI.—AVERAGE BODY AND ORGAN WEIGHTS OF MICE FOLLOWING REPEATED DOSES OF TERIDAX[a]

Teridax Dosage, Mg./Kg./Day	No. of Animals	Initial Body Weight	Autopsy Body Weight	Testes Weight	Seminal Vesicle Weight	Spleen Weight	Adrenal Weight	Kidney Weight	Liver Weight	Heart Weight	Thymus Weight
900	7	19.6	23.8	0.1788	0.0497	0.0895	0.0044	0.458	1.560	0.1157	0.0266
600	12	19.2	25.1	0.1851	0.0548	0.0917	0.0044	0.460	1.614	0.1067	0.0277
300	15	18.8	24.4	0.1946	0.0576	0.0971	0.0051	0.478	1.460	0.1067	0.0257
Controls	15	19.5	25.1	0.1990	0.0577	0.1081	0.0039	0.497	1.558	0.1123	0.0281

[a] In Grams.

Five-Week Oral Subacute Toxicity in Mice.[1]—A five-week, oral, subacute toxicity test was performed for Teridax in male Carworth mice weighing 18–24 Gm. Three groups of 15 mice each were fed the compound by oral syringe once daily, five days a week, for the duration of the experiment. A fourth group of 15 mice was fed the vehicle alone (aqueous 2% gum acacia) as a control. The mortality after five weeks of treatment was as follows:

Group	Substance	Dosage, Mg./Kg./Day	Mortality
1	Teridax	900	8/15
2	Teridax	600	3/15
3	Teridax	300	0/15
4	Controls (2% gum acacia)	...	0/15

At the 900 mg./Kg. dose, two deaths occurred on the second day, one death on the fifteenth and twenty-seventh days, and four deaths on the eighteenth day; at 600 mg./Kg., three deaths on the twenty-fifth day. These deaths have been attributed to the high dosage of compound fed. All animals at the 300 mg./Kg. dosage and in the control group survived.

Just prior to autopsy, blood determinations were made. The total and differential white blood cell counts, red blood cell counts, and hemoglobin values were normal for compound-treated mice and controls (Table V). At autopsy, body weights and weights of the testes, seminal vesicles, spleen, liver, kidneys, adrenals, heart, and thymus were taken; the average organ weights of the drug-fed mice did not differ significantly from the controls (see Table VI). Gross examination indicated no pathological changes. A micropathological study of the liver, heart, spleen, kidney, lung, pancreas, colon, stomach, adrenal, and testis tissues revealed no pathological changes attributable to the administration of the compound.

Twelve-Week Chronic Toxicity in Rats.—Teridax was given orally by stomach tube five days per week, for twelve weeks, to immature female Sprague-Dawley rats. Three groups of ten rats each were fed daily 1,500 mg./Kg., 750 mg./Kg., and 375 mg./Kg. of the compound, respectively. A fourth control group received the vehicle alone (aqueous 0.5% Tween 80).

Mortality after twelve weeks was one of ten at the 1,500 mg./Kg. dose (one rat killed on thirty-seventh day of experiment because of infection), zero of ten at the 750 mg./Kg. dose, zero of ten at 375 mg./Kg. and zero of ten at the control level. The growth rate and mean body weights were normal for compound-fed rats and the controls.

Just prior to autopsy, blood determinations showed normal total and differential white blood cell counts, red blood cell counts, blood sugars, and hemoglobin values in all groups (Table VII). Gross examination showed no pathological changes. Weights of the testes, seminal vesicles, spleen, liver, kidneys, adrenals, heart, and thymus were taken. The mean organ weights of the rats treated with Teridax did not differ from the controls, except for some increases in liver weights at 750 and 1,500 mg./Kg. dose levels, and a decrease in ovarian weight at the 1,500 mg./Kg. dose level (Table VIII). Micropathological study of tissues from these rats chronically fed Teridax showed an absence of cumulative toxicity at all dosage levels.

Repeated Administration to Dogs.—Seven pups were weaned and allowed to grow to a body weight of 4–6 Kg. During this period, the health of the animals was carefully observed and maintained. Inoculations against distemper were made, and deworming procedures were performed. Blood examinations established total and differential leucocyte counts, red cell counts, hemoglobin, and sugar values to be at normal levels. Bromsulphalein liver function tests gave normal values, and urinary excretion patterns for phenosulfonphthalein indicated normal kidney function. The young dogs were then divided into groups according to size and sex. One group received orally 450 mg./Kg. of Teridax, five times per week, and another group of dogs received 450 mg./Kg. of iodoalphionic acid on the same regimen. After twelve weeks of medication at these high dosages, liver and kidney tests indicate normal function in both groups. Blood sugar levels, white blood cell counts, differential counts, red blood cell counts, and hemoglobin values also remained normal.

[1] The authors are indebted to Dr. Frederick Offenkrantz for the histopathological examination of animal tissues, and to Mr. Sidney Neuwirth for the biometric evaluation of data.

TABLE VII.—AVERAGE BLOOD VALUES IN RATS FOLLOWING TWELVE-WEEK CHRONIC TOXICITY TEST

Substance	Dosage, Mg./Kg./Day	No. of Animals	Red Blood Cell Counts Per Cu. Mm., Million	White Blood Cell Counts Per Cu. Mm.	Hemoglobin, Gm./100 Cc.	Blood Sugar, Mg./100 Cc.	Differential Count				
							Neutrophils, %	Lymphocytes, %	Monocytes, %	Basophils, %	Eosinophils, %
Teridax	1,500	9	7.9	20,250	13.9	74	13.4	83.1	2.3	0.1	1.1
Teridax	750	10	8.0	17,737	13.5	79	20.7	76.4	1.6	0.1	1.0
Teridax	375	10	8.6	21,563	13.7	80	11.7	86.4	1.1	0.1	0.8
Controls	...	10	8.1	16,971	14.1	85	13.3	83.4	1.7	0.3	0.6

TABLE VIII.—AVERAGE BODY AND ORGAN WEIGHTS IN RATS FOLLOWING TWELVE-WEEK CHRONIC TOXICITY TEST[a]

Substance	Dosage, Mg./Kg./Day	No. of Animals	Initial Body Weight	Autopsy Body Weight	Ovarian Weight	Uterine Weight	Spleen Weight	Liver Weight	Adrenal Weight	Kidney Weight	Heart Weight	Thymus Weight	Pituitary Weight
Teridax	1,500	9	137.8	213	0.0537	0.365	1.106	10.392	0.0490	1.852	0.685	0.290	0.0134
Teridax	750	10	137.4	218	0.0636	0.424	1.013	9.887	0.0565	1.914	0.702	0.212	0.0122
Teridax	375	10	137.1	214	0.0649	0.392	0.756	8.820	0.0525	1.827	0.677	0.276	0.0140
Controls	...	10	137.3	224	0.0672	0.437	0.883	8.988	0.0510	1.933	0.711	0.214	0.0153

[a] In Grams.

DISCUSSION

A comparison of Teridax with iodoalphionic acid in dogs has clearly demonstrated this substance to produce a sharper outline of the gall bladder in excellent contrast with adjacent tissues. Comparisons show 150 mg./Kg. of Teridax to produce opacity and definition equal to 300 mg./Kg. of iodopanoic acid. The three contrast agents were studied in the same dogs to minimize individual variation in arriving at estimates of gall-bladder radiopacity. Bile ducts were seen following Teridax in two dogs; no ducts were visible after iodopanoic acid or iodoalphionic acid.

Following oral Teridax, excellent visualization has been obtained in clinical studies with normal human subjects, and in the routine examination of patients for gall-bladder disease (7). A good parallel has been found between the gall-bladder opacity and definition, colon opacity, absence of gastrointestinal irritation observed in dogs, and results obtained in man. The similarity between gall-bladder opacity and definition found in man, and that seen in dogs, has been cited by Jones, *et al.* (3), and Epstein, *et al.* (4). Shapiro, and Weinberg (7) have shown in man that Teridax does not produce the early and undesirable opacity of the colon commonly observed after iodopanoic acid (8–11). In conformity with these findings, metabolic studies in dogs and man indicate that Teridax, like iodoalphionic acid, is excreted primarily by the kidneys, and iodopanoic acid is excreted in the feces (12).

Freedom from emetic reactions and from gastric irritation was noted in the studies with dogs following repeated Teridax doses of 450 mg./Kg./day. Chronic toxicity studies with mice, rats, and dogs at daily oral dosages which were several times the dosage required for human subjects indicate a low toxicity for the compound. The low toxicity of Teridax also is apparent from observations recorded during the routine application of Teridax in human diagnostic procedures. The data demonstrate the safety of Teridax and a low incidence of side effects in clinical usage (7).

SUMMARY

Teridax (triiodoethionic acid), a new oral cholecystographic medium, was evaluated in non-anesthetized, trained dogs. At 100 mg./Kg., Teridax visualized the gall bladder more distinctly than 300 mg./Kg. iodoalphionic acid. The new compound at 150 mg./Kg. was as effective as 300 mg./Kg. of iodopanoic acid. Maximal opacity occurred between eight to ten hours with both Teridax and iodoalphionic acid. Very little Teridax appeared in the colon of dogs.

The safety of Teridax is indicated by acute toxicity data obtained in mice, rats, guinea pigs, and dogs; the compound was well tolerated in animals (mice, rats, and dogs) receiving repeated massive doses. In dogs, no emetic reactions occurred, nor was there any evidence of gastric irritation. Pharmacodynamic tests with anesthetized dogs have not shown any deleterious effects upon respiration or the cardiovascular system. The available clinical data indicate a close parallel between the gall-bladder opacity and definition, colon opacity, absence of gastrointestinal irritation, and the safety of Teridax in laboratory animals and in man.

REFERENCES

(1) Papa, D., Ginsberg, H. F., and Lederman, I., Division of Medical Chemistry, American Chemical Society Meeting, Atlantic City, New Jersey, September 14, 1952; *J. Am. Chem. Soc.* In Press.

(2) Hoppe, J. O., (1951). Unpublished data.

(3) Jones, G. E., Grohowski, A. S., Robertson, H. D., Ramsey, G. H., Schilling, J. A., and Strain, W. H., *Radiology*, **51**, 225(1948).

(4) Epstein, B. S., Natelson, S., and Kramer, B., *Am. J. Roentgenol.*, **56**, 201(1946).

(5) Neuhaus, D., Christman, A. A., and Lewis, H. B., *Proc. Soc. Exptl. Biol. Med.*, **78**, 313(1951).

(6) Litchfield, J. T., and Wilcoxon, F., *J. Pharmacol. Exptl. Therap.*, **95**, 99(1949).

(7) Shapiro, R. S., Scientific Exhibit, 38th Annual Meeting Radiological Society of North America, Cincinnati, Ohio,

December 7–12, 1952; Weinberg, C. Richard, "Preliminary Evaluation of a New Cholecystographic Medium—'Teridax,'" (to be published).
(8) Christensen, W. R., and Sosman, M. C., *Am. J. Roentgenol.*, **66**, 764(1951).
(9) Everett, E. F., and Rigler, L. G., *Radiology*, **58**, 524 (1952).
(10) Lowman, R. M., Stanley, H. W., Evans, T. S., and Mendillo, J. C., *Gastroenterology*, **21**, 254(1952).
(11) Abel, M. S., Lomhoff, I. L., and Garcia, C. V., *Permanente Foundation Med. Bull.* **10**, 95(1952).
(12) Perlman, P. L., Kosinski, R., and Sutter, D., "Studies on the Absorption and Excretion of a New Cholecystographic Agent, Teridax," (to be published).

The Influence of Emulsifying Agents on the Antiseptic Activity of Certain Dermatologic Preparations*

By J. A. WOOD† and L. WAIT RISING‡

Five series of oil-in-water emulsions and four series of water-in-oil emulsions containing antiseptics were tested by the F. D. A. agar cup-plate method to determine the effect of different emulsifiers on their antiseptic values. The results indicated that no one emulsifier could be recommended for all antiseptics, but, in general, oil-in-water emulsions appeared to be more effective vehicles for antiseptics than water-in-oil emulsions.

MUCH OF THE empiricism in dermatologic medication is being removed by the rapid accumulation of scientific knowledge concerning the proper formulation of topical applications. It is now recognized, for example, that emulsions make ideal vehicles for skin medication in many instances. As a result of this recognition many investigations have been conducted in order to determine the most suitable physical properties for these highly specialized preparations.

An important factor in the control or variation of the physical properties is the emulsifier used. Since these agents are frequently active chemicals their presence might directly influence to an important degree the therapeutic effect of the medicinal ingredients. If such were the case a change in the nature or composition of the emulsifier might produce a significant alteration in the therapeutic efficiency of the application despite the fact that physically the emulsion was correct.

This paper describes an investigation designed to answer, in part, the question of possible change in therapeutic effectiveness due to substitution of emulsifiers.

EXPERIMENTAL

Two standard emulsion formulas were selected for study. One was an oil-in-water base; the other was a water-in-oil base. A series of 15 antiseptic emulsions was made from each. The preparations in each series varied only in the antiseptic incorporated, which was different for every emulsion. This procedure produced 30 emulsions containing separate and distinct antiseptics, 15 of which were oil-in-water and 15 water-in-oil preparations. They were tested for their relative antiseptic abilities by the standard F. D. A. agar cup-plate method, using the unmedicated bases for controls and *Staph. aureus* as the test microorganism.

With these data as a reference point it was practical to vary the emulsifier portion of the two base formulas in order to determine what influence, if any, the change would have on the relative antiseptic values of the emulsions as shown by the F. D. A. procedure.

Four additional series of oil-in-water and three of water-in-oil preparations were made. The sequence of antiseptic materials was kept. The only ingredient change was the substitution of a different emulsifier in the basic formulas for each series. Testing of the seven added series by the agar cup-plate method gave comparative data on the influence of five different oil-in-water emulsifiers and four water-in-oil emulsifiers on the release of antiseptic for diffusion through the seeded media. Table I shows the basic formulas and Table II shows antiseptics used together with the results of the tests.

DISCUSSION

Examination of the nine basic formulas used will show an apparent lack of consistency in maintaining the quantitative ratio of the ingredients throughout the series. It was necessary to change the amounts incorporated in order to insure constancy of physical properties in all the preparations.

One of the disturbing results was the failure of zones of inhibition to develop with the sulfonamides, chrysarobin, and tyrothricin. Subsequent investigation with sulfathiazole brought out the fact

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TABLE I.—EMULSION BASE FORMULAS

Components	Oil-in-Water Bases[a] 1	2	3	4	5
Methylparaben	0.25	0.25	0.25	0.25	0.25
Propylparaben	0.15	0.15	0.15	0.15	0.15
Glycerin	120	53.4	70.6	66	77.6
Stearyl alcohol	250	113.3	147.2	137	165.7
White petrolatum	250	113.3	147.2	137	165.7
Water	370	600	600	500	500
Sodium lauryl Sulfate	10	...	...	...	...
Tegin[b]	...	120	...	...	...
Tegin P[c]	...	...	35	...	...
Span 60	...	...	...	100	...
Tween 60	...	...	...	60	...
Triethanolamine	...	...	...	...	30
Stearic acid	...	...	...	...	61

Components	Water-in-Oil Bases[a] 6	7	8	9
Stearyl alcohol	15	12	14	15
White wax	40	32	38	39
Wool fat	75	60	72	73
White petrolatum	365	291	384	356
Water	500	405	478	487
Cholesterol	5	...	...	...
Protegin X[d]	...	200	...	...
Arlacel C[e]	...	...	50	...
Wool alcohols	...	...	...	30

[a] All quantities are recorded in Gm.
[b] Glyceryl monostearate (self-emulsifying).
[c] Propylene glycol monostearate (self-emulsifying).
[d] Cholesterols extracted from lanolin.
[e] Arlacel 83 (sorbitan sesquioleate).

TABLE II.—AGAR CUP PLATE ZONES OF INHIBITION FOR EACH MEDICATED PREPARATION

	Medicament	Concentration[d]	Zones[a] of Inhibition in Mm. for Each Base 1	2	3	4	5	6	7	8	9
	Control	..	0	0	0	0	0	0	0	0	0
A.	Ammoniated mercury	5	9	10	9.75	9.25	8	7	8.5	9.5	9
B.	Iodoform	10	4	0	1	1	0	2	8	3.5	2.5
C.	Benzoic and salicylic acids	12:6	9	10.25	[b]	8	10.75	[b]	7	7.5	7.25
D.	Zephiran chloride	1 in 500	0.75	3	5.5	4	2.5	[c]	[c]	0	[c]
	Sulfathiazole	5	0	0	0	0	0	0	0	0	0
	Sulfathiazole sodium	5	Some inhibition but no clear zones								
	Chrysarobin	6	0	0	0	0	0	0	0	0	0
	Sulfamerazine	5	0	0	0	0	0	0	0	0	0
E.	Resorcinol	6	4	3	3.75	5	4.75	[b]	0	[b]	[b]
F.	Mercuric oxide (yellow)	2	8	10	8.5	10.5	7.25	6.5	6.75	8	6.5
G.	Phenol	2	3.25	2.5	1	1	4	[c]	[c]	2.5	[c]
	Ustilagic acid	0.5 mg./Gm.	0	0	0	0	0	0	0	0	0
	Tyrothricin	0.5 mg./Gm	0	0	0	0	0	0	0	0	0
H.	Iodine and potassium iodide	4:4	25.5	27.5	29	8	16	15	11	11	12.5
I.	Crystalline penicillin G	500 u./Gm.	14	13.5	13.5	14	11.5	11.5	11.5	21	10

[a] Measured from edge of cup to perimeter of zone.
[b] Incompatible.
[c] No ointments for testing.
[d] Figures represent per cent unless otherwise indicated.

that when a nutrient media containing a lower concentration of beef extract and peptone was used (3 Gm. and 5 Gm., respectively, in place of the 5 Gm. and 6 Gm. called for in the agar cup-plate method) 6-mm. zones developed. Presumably similar results could have been obtained with the others if different media had been used. This serves to confirm the contention that data gathered on the basis of the agar cup-plate procedure are valid only for a particular set of conditions.

SUMMARY AND CONCLUSION

Five series of oil-in-water emulsions and four series of water-in-oil emulsions containing antiseptics were tested by the F. D. A. agar cup-plate method to determine the effect of different emulsifiers on their antiseptic values; 135 emulsions were examined.

The data collected showed that the emulsifying agent can, at times, play an important role in improving or reducing the efficiency of release of the antiseptic.

No one emulsifier can be recommended for all antiseptics. Proper choice depends on the particular antiseptic to be used.

In general, under the conditions of this study, oil-in-water emulsions were more effective vehicles for antiseptics than were water-in-oil emulsions, although there were some notable exceptions.

The Metabolism of Prantal[1] in Dogs and Methods for the Quantitative Estimation of Prantal in Biological Material*

By PRESTON L. PERLMAN, CAROL BONSAL JOHNSON, and ROBERT E. KOSINSKI

Methods are described for the estimation of Prantal in biological fluids and tissues. From studies on dogs it was found that the compound is well absorbed following oral administration, and following oral, intravenous, or intramuscular administration, the original quaternary cation, as demonstrated by countercurrent distribution and isolation, is eliminated by both the kidney and intestine. No evidence of storage was found although a significant per cent of the administered drug could not be accounted for.

THIS INVESTIGATION was carried out to determine the extent of urinary and fecal excretion, blood levels, and the chemical nature of the urinary excretion products following the administration of the parasympathetic blocking agent, Prantal, to mature dogs. In addition, the pattern of excretion was determined following both oral and intravenous administration. Certain observations on the pattern following intramuscular administration are also presented.

A preliminary report (1) on Prantal metabolism has already appeared. The present study deals with the detailed aspects of the study.

METHODS

The method of estimation is based on a modification of the technique of Brodie, *et al.* (2), and Cochin and Woods (3) for the estimation of organic bases. Prantal, a quaternary compound, is soluble in water but not to any appreciable extent in chloroform. The bromcresol purple-Prantal complex (*p*H 7.0) is soluble in chloroform but not in water. Hence, the chloroform soluble dye-drug complex, which forms stoichiometrically in the presence of excess aqueous dye solution is prepared and the chloroform layer containing the dye-drug complex removed. The dye component of the complex is then extracted into aqueous alkali and the color intensity of the alkaline dye solution quantitatively measured colorimetrically.

Reagents

Phosphate Buffer.—Two parts 1.5 *M* Na_2PO_4 are added to one part 1.5 *M* KH_2PO_4 and the *p*H of the mixture is adjusted to exactly *p*H 7.0 (glass electrode) by the addition of the appropriate phosphate solution. The adjusted buffer solution is filtered, saturated with chloroform and stored in a Pyrex glass-stoppered container.

Bromcresol Purple Solution.—An amount of 0.4 Gm. bromcresol purple (Eimer and Amend) is dissolved in 10 ml. ethanol, 6.4 ml. 0.1 *N* NaOH added, the solution is transferred to a 500-ml. volumetric flask and the contents brought to volume with distilled water. The dye solution is then shaken with one 100-ml. portion of chloroform in order to saturate the aqueous dye solution with the chloroform and to remove any colored, chloroform-soluble impurities which may be present in the original dye. The chloroform saturated dye solution is centrifuged to clarify the solution and the clear, purple aqueous solution is stored in a glass-stoppered bottle.

Chloroform (C. P.).—Saturate with distilled water before using.

0.5 *N* NaOH.—Saturate with chloroform.

Prantal Standard Solution.—A *stock solution* is prepared by dissolving 100 mg. Prantal in 1.0 L. of distilled water in a 1.0 L. volumetric flask. A *working standard* is obtained by preparing a 1:10 dilution of the stock solution with distilled water, thereby producing a final concentration of 10 μg./ml.

Procedures

Colorimetric Estimation of Prantal.—Duplicate 1–6 ml. aliquots of the Prantal-containing solutions (standards and unknowns) and containing 10–50 μg. Prantal are delivered into 35-ml. glass-stoppered test tubes. Distilled water blanks are also prepared at this time.

To each tube add 2 ml. phosphate buffer, 4.0 ml. dye solution, and 12.0 ml. chloroform. The stoppers are moistened with water, securely inserted in the tubes, and the tubes mounted on a mechanical platform rocker. The platform rocker used in these studies provides seven full oscillations per minute, each oscillation being 35° from the horizontal. Rocking is carried out for thirty minutes. The tubes are then removed from the rocker, the stoppers removed, the tubes briefly centrifuged to provide clear separation of the two layers, and a 10-ml. chloroform layer aliquot from each tube transferred to a clean tube. Two ml. 0.5 *N* NaOH are added to each of the tubes containing the 10 ml. $CHCl_3$ extract, the stoppers moistened with $CHCl_3$ and firmly seated in the tubes. The tubes are mounted on a mechanical shaker which provides vigorous shaking of 140 full strokes per minute, each stroke covering a 100° arc from the vertical. Shaking is carried out for five minutes and then the tubes are briefly centrifuged. A minimum of 1.0 ml. of the top layer (aqueous alkali) is pipetted from each tube into colorimeter tubes (1.0 ml. minimum volume) and the optical

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[1] Registered trade mark of the Schering Corporation for N,N-dimethyl-4-piperidylidene 1,1-diphenylmethane methyl sulfate.

densities measured at 595 mμ with a Coleman Junior Spectrophotometer. The instrument is zeroed with distilled water. (It was noted that the readings decreased slightly after the tubes stood for a time; an optical density decrease of 0.01 in fifteen minutes being observed.)

Recovery of the drug from a distilled water solution demonstrated a recovery of 100 ± 1.0% of added Prantal.

A plot of the optical densities, corrected for blank, against drug concentration is linear, passes through the origin, and conforms closely to the Lambert-Beer law within the range of 5–50 μg.

For the analyses of "unknown" solutions, aqueous blanks and two concentrations of standard Prantal solution are always run because of slight variations observed in day to day readings. These variations appear to be induced by certain temperature and timing factors.

Urine.—Urine is collected under a small amount of heptane. The urine volume is measured and a filtered aliquot no larger than 5 ml. is used for analysis. The aliquot volume is selected so that it contains an amount of Prantal intermediate between the standard concentrations and is determined by an approximate preliminary analysis.

Urine blanks were run on each dog before the animal was used in an experiment. The urine blanks were found to vary from dog to dog, but not for any one dog from day to day. With a 5-ml. urine sample obtained from a series of dogs, blank values with an optical density in the range of 0.15–0.50 were obtained. There was also noted a direct relationship between volume of urine and optical density of the blank value.

Recovery experiments showed a consistent recovery of 98% of added drug after correction of the optical density readings for the blank for the aliquot size used.

Feces.—The total daily feces are thoroughly mixed and weighed and duplicate 8.0 Gm. samples transferred to small, stainless steel Waring Blendor cups. Seventy ml. 1.0 *N* HCl and 5 ml. distilled water are added. The mixture is homogenized for five minutes, the volume adjusted to 80 ml. and the entire material centrifuged at 2,000 r. p. m. for two hours to yield a clear or almost clear supernatant.[2] The supernatant is then filtered. The volume analyzed is selected to contain an amount of drug intermediate between the standard concentrations employed. Sufficient 6 *N* NaOH is added to produce a *p*H of 7 (Hydrion paper) and the total volume brought to 6.0 ml. with distilled water before proceeding with the analysis. A sample of the filtrate no greater than 5 ml. is used for the estimation of Prantal.

Before experiments were carried out on any dog, the fecal blank for that dog was initially determined. This blank, like that for the urine, varied from animal to animal, but did not vary significantly for a given dog.

Recovery experiments with pure Prantal added to feces revealed that 85% of the added drug was consistently obtained. Hence, all experimental data on Prantal content of feces have been corrected for the 85% recovery.

[2] Attempts were made to precipitate or extract interfering substances using a variety of reagents but in all cases the drug was carried down with the precipitate and could not be satisfactorily recovered even with successive washing of the precipitate.

TABLE I.—PER CENT OF PRANTAL EXCRETED BY DOGS IN URINE AND FECES FOLLOWING A SINGLE INTRAVENOUS DOSE, 7.5 MG./KG.

Collection Period, Days	Dog No. IV-1 Urine[a]	IV-2 Urine[b]	IV-2 Feces	V Urine	V Feces	VI Urine	VI Feces	XV-1 Urine	XV-1 Feces	XV-2d Urine	XV-2d Feces	XV-3d Urine	XV-3d Feces
0–0.5	9.3	11.1	N. F.[c]	14.6	N. F.	12.7	N. F.	...	N. F.	...	N. F.	...	N. F.
0.5–1	15.1	9.9	N. F.	13.3	5.2	14.9	1.6	16.2[e]	0	13.8[e]	10.4	17.3[e]	4.4
1–2	4.1	4.9	16.8	3.4	1.7	4.2	9.9	2.3	10.2	1.6	12.7	3.3	12.7
2–3	2.5	2.4	2.5	2.4	0.6	2.8	10.2	0	0	0	2.5	0	0
3–4	1.7	0	0	1.1	0.9	2.4	3.3	0	0	0	0	0	0
4–10	0	0	0	3.2	1.2	7.3	2.3	0	0	0	0	0	0
10–12	0	0	0	1.0	0	1.1	0	0	0	0	0	0	0
12–13	0	0	0	0	0	2.1	0	0	0	0	0	0	0
Urine total (Days 0–4)	32.7	28.3		34.8		37.0		18.5		15.4		20.6	
Feces total			29.3		8.4		25.0		10.2		25.6		17.1
Total urine and feces			57.6		43.2		62.0		28.7		41.0		37.7

[a] Feces excretion not determined.
[b] Only days 0–3 studied.
[c] N. F. signifies no feces eliminated.
[d] This dose was administered seven days after previous one.
[e] Total twenty four-hour collection. Average urine excretion = 26.8%.
Average feces excretion = 19.3%. Average urine and feces = 46.1%.

Blood.—Ten ml. blood collected in a heparin-wetted syringe are delivered into a centrifuge tube. An equal volume of distilled water is added to the blood, the contents thoroughly mixed and permitted to stand fifteen minutes. Centrifuge at 2,000 r. p. m. for approximately two hours and pipette a 10-ml. aliquot of the supernatant (equivalent to 5 ml. whole blood) into a 50-ml. glass-stoppered centrifuge bottle. Adjust the volume to 16 ml. with distilled water and add the following reagents: 4 ml. phosphate buffer, 8 ml. dye solution, and 30 ml. chloroform. The already described procedure for the estimation of Prantal is carried out on a 25-ml. aliquot of the chloroform layer.

The blood blanks were of the same order as the blank values obtained from aqueous blanks. Recovery experiments showed 100% recovery in the range of 10 μg. Prantal/10 ml. whole blood. The limit of absolute estimation by this procedure was 1.0 μg. in a 5.0-ml. blood sample (0.2 μg./ml.).

RESULTS

Rate of Excretion Following a Single Intravenous, Oral, and Intramuscular Dose.—Estimations of the amount of bromcresol purple reacting substances excreted in urine and feces were carried out following single intravenous or oral doses of the compound to a group of dogs maintained in individual metabolism cages. The results are given in Tables I and II. For ease of comparison, the per cent of drug excreted in the urine during the first four days following the single administration has been totaled. The total per cent eliminated in the feces is cumulated for the entire experimental period. The reason for this method of summarizing the data is based on the fact that only small amounts of the drug are excreted in the urine after the fourth day. However, the magnitude of Prantal elimination in the feces is more variable in the sense that in some of the experiments the major portion is excreted within a few days after administration, in other experiments only after several days have elapsed.

A comparison of the excretion data following a single intravenous dose (7.5 mg./Kg.) (Table I) with that following a single oral dose (37.5 mg./Kg.) (Table II) suggests greater consistency, i. e., less variability in the per cent eliminated in the urine and feces with the intravenous route. The rate of elimination and the total per cent eliminated appeared to be about the same. In the seven instances in which urinary excretion was measured (Table I) the per cent of the total dose eliminated in the first four days was 15.4–37.0% (average 26.8%). The fecal excretion, determined in six instances, showed more variability (average 19.3%) and the total recovery (urine plus feces) in all dogs studied ranged from 28.7–62.0% (average 46.1%).

TABLE II.—PER CENT OF PRANTAL EXCRETED BY DOGS IN URINE AND FECES FOLLOWING A SINGLE ORAL DOSE, 37.5 MG./KG.

Collection Period, Days	Dog No. III-a U.[a]	III-b U.	IV-a U.	IV-b U.	IV-b F.	IV-c U.	IV-c F.	V-a U.	V-a F.	V-b U.	V-b F.	VII U.	VII F.
0–0.5	...	9.8	26.8	...	N. F.[b]	...	0.0	41.7	N. F.	39.0	N. F.	0.8	N. F.
0.5–1	52.0	18.2	16.7	4.8	16.8	4.8	0.0	12.7	N. F.	6.9	N. F.	3.9	N. F.
1–2	1.0	1.1	0.2	0.9	2.5	0.7	2.0	1.3	N. F.	2.6	N. F.	3.2	17.2
2–3	0.1	0.2	0.5	0.7	0.0	0.7	1.0	0.4	0.3	0.3	N. F.	0.4	0.4
3–4	0.1	0.1	0.2	0.6	0.0	0.6	3.5	0.2	0.1	0.3	34.5	0.2	0.3
4–10	0.0	0.0	0.6	1.2	0.0	0.4	0.0	1.0	0.0	0.5	0.4	0.5	3.7
10–13	0.0	0.0	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Urine total (Days 0–4)	53.2	29.4	44.4	7.0		6.8		56.3		49.1		8.5	
Feces total					19.3		6.5		0.4		34.4		17.9
Total urine and feces[f]				26.3		13.3		56.7		83.5		26.4	

XVIII U.	XVIII F.	XII-1 U.	XII-1 F.	XII-2[c] U.	XII-2[c] F.	XII-3[d] U.	XII-3[d] F.	XIV-1 U.	XIV-1 F.	XIV-2[c] U.	XIV-2[c] F.	XIV-3[d] U.	XIV-3[d] F.	Collection Period, Days
...	...	...	...	...	...	...	...	...	...	...	...	...	...	0–0.5
N. U.	N. F.	6.1	N. F.	32.9[e]	0.0	N. U.	N. F.	N. U.	N. F.	31.1	0.0	30.6	5.5	0.5–1
0.7	69.1	3.5	11.3	0.8	3.3	5.8	26.3	56.8	24.6	16.0	1.6	0.9	13.2	1–2
0.3	3.9	1.4	4.5	0.1	1.0	0.4	2.0	N. U.	0.4	N. U.	0.7	0.4	12.6	2–3
0.2	1.9	0.0	0.0	0.2	2.0	0.0	0.6	0.0	0.0	0.3	0.1	0.0	0.0	3–4
0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4–10
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10–13
1.2		11.0		34.0		6.2		56.8		47.4		31.9		Urine total (Days 0–4)
	74.9		15.8		6.3		28.9		30.0		2.4		31.3	Feces total
	76.1		26.8		40.3		35.1		86.8		49.8		63.2	Total urine and feces[f]

[a] Feces excretion not determined.
[b] N. F. signifies no feces eliminated.
[c] This dose administered 9 days after previous dose.
[d] This dose administered 12 days after previous dose.
[e] Total twenty-four-hour collection for animals XII to XIV-3, inclusive.
[f] Combined average exclusive of Dogs III-a, III-b and IV-a.
Average urine excretion = 29.6%. Average feces excretion = 22.7%. Average urine and feces = 49.1%.

Examination of the data obtained from dogs receiving a single oral dose (Table II) reveals that the magnitude of drug excretion in the urine ranged from 1.2 to 56.8% (average 29.6%) and the fecal excretion from 0.4 to 74.9% (average 22.7%). The total excretion (urine plus feces) ranged from 13.3 to 86.8% with a mean per cent of 49.1. It also should be noted that even for a single animal there appears to be significant variations in elimination patterns after the administration of single drug doses.

It is not possible to assess more carefully the differences, if any in fact do exist, in excretion pattern between the oral and intravenous routes due to the fact that the doses for each route were different. The dose given intravenously was 7.5 mg./Kg. and for the oral studies 37.5 mg./Kg. In an attempt to rule out the contribution of dose, one dog was given Prantal intramuscularly in saline at a dose of 37.5 mg./Kg. The excretion pattern is given in Table III. These data indicate that fecal elimination of Prantal, even with doses of 37.5 mg./Kg., accounts for a significant percentage of the administered drug. It also indicates that the fecal elimination obtained with the oral route of administration is most probably not due to a lack of gastrointestinal absorption of the compound but most likely due to a reabsorption of the drug from the circulatory system back into the gastrointestinal tract for elimination. It is also of interest to note that the per cent recovery obtained with the intramuscular route of administration was the highest ever observed for any animals studied regardless of route employed.

TABLE III.—PER CENT OF PRANTAL ELIMINATED IN URINE AND FECES FOLLOWING A SINGLE INTRAMUSCULAR DOSE, 37.5 MG./KG., TO A DOG

Collection Period, 24 Hr.	Dog No. XV	
	Urine	Feces
1st	19.7	1.2
2nd	4.1	66.7
3rd	1.7	2.5
4th	1.0	3.3
5th	0.9	1.9
Total	27.4	75.6
Combined total	103	

Blood Levels Following a Single Intravenous or Oral Dose.—The data on urinary and fecal elimination revealed that although a considerable amount of bromcresol purple reacting material was eliminated in both the urine and feces, a significant amount (approximately 50%) of the drug could not be accounted for in certain of the animals. This fact raises the question of the fate of the unaccounted-for drug. Several experiments were carried out to ascertain the amount of Prantal circulating in the blood stream following intravenous administration and the results revealed that less than 1.0 μg./ml. of plasma was present thirty minutes after the intravenous administration of the material. No measurable concentration was found at several intervals following oral administration. These results indicate that either extensive storage and/or dilution in body tissues and/or destruction of the compound was occurring.

To obtain evidence of storage, experiments are now being conducted in which representative tissues are being analyzed for their ability to store and/or transform the administered compound. The results of these studies will be reported in a future publication.

Rate of Excretion Following Serial Oral Administration.—Experiments were carried out in which the daily Prantal excretion in urine and feces were estimated following the administration of seven successive, daily oral doses of the drug (Table IV). It is to be noted that during the period of drug administration the daily urine excretion was relatively constant for each dog. Within the twenty-four hour period following cessation of treatment the excretion level dropped sharply and by the fifth post-treatment day no drug could be detected in the urine or feces. These results are interpreted to indicate that the drug is eliminated, and possibly destroyed, at constant rates; they also suggest that little storage occurred. If storage was taking place it might be expected that "leakage" of the drug would be observed for some time following cessation of the treatment.

TABLE IV.—MG. PER DAY AND TOTAL PER CENT OF PRANTAL EXCRETED BY DOGS IN URINE AND FECES FOLLOWING A SEVEN-DAY SERIES OF ORAL DOSES, 37.5 MG./KG. PER DAY

Collection Period, Days	Dog XVI		Dog XVII	
	Urine	Feces	Urine	Feces
	Mg./Day		Mg./Day	
0–1	17.8	N. F.	5.5	174.2
1–2	63.3	14.7	11.4	4.1
2–3	48.7	83.2	24.2	N. F.
3–4	61.0	N. F.	29.7	152.0
4–5	75.7	224.4	36.8	N. F.
5–6	62.4	108.5	47.1	304.2
6–7	77.7	150.8	92.7	21.2
Last dose				
7–8	22.1	44.7	18.6	0
8–9	6.2	0	5.5	0
9–10	5.3	0	8.9	N. F.
10–11	2.7	N. F.	5.2	1.2
11–12	0	0	0	0
Urine total	442.9[a] (15.5%)		285.6[b] (8.8%)	
Feces total	626.3 (21.9%)		656.9 (20.1%)	
Combined total	1069.2 (37.4%)		942.5 (28.9%)	

[a] The total dose given to this dog was 2,849 mg.
[b] The total dose given to this dog was 3,255 mg.

Effect of Continued Oral Administration on Excretion Pattern.—It has already been demonstrated above that the kidneys and intestine are major pathways for Prantal elimination. The data indicate that in general one-half the total eliminated quaternary compound is excreted with the feces and one-half in the urine although extremes for each route also have been observed in individual animals. What the effect of continued exposure to Prantal would have on the elimination pattern was considered of interest because examinations of the excretion pattern of two dogs receiving chronic oral treatment of a very high dose (120 mg./Kg./day) revealed (Table V) a predominant fecal excretion. This might be considered to be a

reflection of the extremely high dose level of Prantal being administered or, alternatively, an "adaptive" adjustment of the dogs to continued medication. This adaptation could be either with the pathway of absorption or elimination.

TABLE V.—PER CENT OF PRANTAL EXCRETED IN URINE AND FECES BY CHRONICALLY TREATED DOGS[a]

Treatment Period, Days	Dog 1310		Dog 1311	
	Urine	Feces	Urine	Feces
223	5.0	11.4	3.4	84.0
224	4.2	31.2	3.2	61.0
225	6.0	48.3	N. S.[c]	N. S.
226	5.2	34.7	N. S.	N. S.
Mean %[b]	5.1	31.4	3.3	72.5

[a] Dose schedule is 60 mg./Kg. daily at 10:00 a. m., 60 mg./Kg. daily at 4:00 p. m., Monday through Friday; Saturday and Sunday 60 mg./Kg. at 10:00 a. m. only.
[b] Calculated on basis of 120 mg./Kg./day.
[c] N. S. indicates "not studied."

To test this hypothesis one dog who had shown a predominant urinary pathway of elimination was subjected to a series of Prantal doses at 37.5 mg./Kg. and 120 mg./Kg. orally and then with 37.5 mg./Kg. intramuscularly. The details of this regimen are given in Table VI.

TABLE VI.—PER CENT OF PRANTAL EXCRETED IN URINE AND FECES BY A DOG RECEIVING THE DRUG VIA THE ORAL AND INTRAMUSCULAR ROUTES

Regimen	Expt. No.	Total Dose, %	
		Urine	Feces
Single oral dose, 37.5 mg./Kg.	1[a]	56.8	25.0
	2	47.4	2.4
	3	31.9	32.5
Daily oral dose, 120 mg./Kg.	4[b]	0.6	0.1
		1.9	13.7
		...	62.5
		Contd. same treatment for 5 more days	
		2.6	61.7
Daily oral dose, 37.5 mg./Kg.	5[a]	3.7	73.8
		8.5	46.5
		2.6	75.9
10-day rest period here			
Single oral dose, 37.5 mg./Kg.	6	5.0	19.9
6-day rest period here			
Single i. m. dose, 37.5 mg./Kg.	7	27.4	75.6

[a] In this group of three experiments the excretion of each dose permitted to go to completion before administering next dose.
[b] The figures for this experiment represent per cent daily dose and not total dose.

From the data it is observed that the animal exhibited a predominantly fecal elimination at the 120 mg./Kg. dose as well as during the 37.5 mg./Kg. dose which followed. That this apparent "shift" from the first 3–37.5 mg./Kg. doses was probably not due to an adaptation on the part of the absorption mechanism is indicated by the fact that the same predominantly fecal elimination was obtained when 37.5 mg./Kg. of the compound was administered intramuscularly.

It should be mentioned that although there appears to be evidence of a shift from a predominantly urinary elimination to a predominantly fecal one, the absolute amount of compound eliminated via the kidney alone shows that the amount of drug in the blood stream following either oral or i. m. administration should be adequate for eliciting a pharmacological response at least equal to that obtained with the intravenous dose.

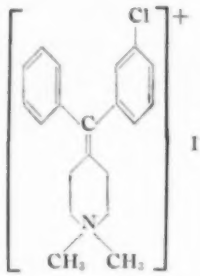
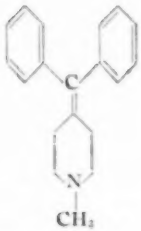
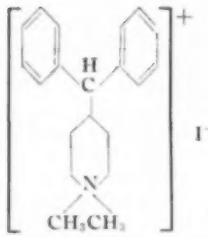
Nature of Urinary Excretion Product.—It was deemed of interest to ascertain the chemical nature of the excretion products following the administration of Prantal to dogs. Toward this end, a 9-plate countercurrent distribution in an all-glass Craig distribution machine was carried out. It was found that 0.2 *M* aqueous potassium acid phthalate (*p*H 5.5) as the aqueous phase and chloroform as the organic phase yielded a satisfactory apparent distribution coefficient (K_a = 1.13). The distribution coefficient with this solvent pair does not appear to depend primarily upon the *p*H of the phthalate solution but appears instead to depend upon the phthalate molecule *per se*. This was indicated by the fact that substitution of other buffers (phosphate and acetate) at the same *p*H for the phthalate solution produced distributions strongly favoring the aqueous phase. An actual distribution of pure Prantal in the phthalate-$CHCl_3$ mixture was carried out and compared with the distribution of a 5-ml. urine aliquot obtained four hours after a dog received a single oral dose of the compound. Close agreement between the two curves was obtained. That the distribution is not dependent upon the nature of the anionic component of the molecule, i. e., methyl sulfate, was demonstrated by the fact that the methyl iodide quaternary of the parent amine yielded a distribution curve identical with that of the quaternary methyl sulfate.

Although the countercurrent distribution data suggested that the bromcresol purple reacting material in the urine was indeed the quaternary moiety of Prantal, it was realized that other organic bases would react with the bromcresol purple in the same manner as Prantal. If these substances had identical distribution coefficients with Prantal, the countercurrent distribution data would lose significance. To delimit this possibility certain compounds related to Prantal were studied for their distribution coefficients in the phthalate-$CHCl_3$ system. The results are given in Table VII.

From these data the conclusion can be drawn that saturation of the double bond (Sch 1612), loss of quaternary function (Sch 1471), and substitution in one of the benzene rings (Sch 2001) would produce significant changes in the distribution pattern compared with the Prantal quaternary cation.

Final proof of identity of the bromcresol purple reacting material in the urine was obtained by the isolation of the quaternary cation from urine as the Reinecke salt and its subsequent conversion to the known methiodide according to the procedure described by Zaimis (4). From a urine sample containing the suspected Prantal quaternary cation, the crystalline methyl iodide derivative was prepared. The physical constants of this derivative, compared to those of an authentic sample of Prantal methyl iodide are as follows: m. p. (isolated)

TABLE VII.—APPARENT DISTRIBUTION COEFFICIENTS, *Ka*, OF CERTAIN COMPOUNDS RELATED TO PRANTAL METHYL SULFATE

Compound	Apparent Distribution Coefficient, *Ka*
Sch 2001 	*Ka* = 1.80
Sch 1471 	*Ka* = 41.6
Sch 1612 	*Ka* = 0.41

258–260°, (authentic) 258–260°, mixed m. p. 258–260°; C, H, N (isolated) 58.7, 5.6 and 3.7%, respectively, C, H, N, (theoretical) 59.3, 6.0 and 3.5%, respectively. These data appear to confirm the nature of the excreted quaternary moiety as being identical with the administered one.

SUMMARY AND CONCLUSIONS

1. A quantitative, colorimetric micro-method for the determination of the parasympathetic blocking agent, Prantal, in pure solution or in biological materials has been developed.

2. It has been demonstrated that the drug is well absorbed following the oral administration of Prantal to dogs and that following oral, intravenous or intramuscular administration, the Prantal quaternary cation is eliminated by both the kidney and the gastrointestinal tract.

3. There appears to be no significant differences in the rate or degree of urinary or fecal elimination when the oral and intravenous routes are compared.

4. On an average, 50 per cent of the administered dose is eliminated regardless of route of administration. Of this material, slightly more than half is eliminated in the urine, the remainder in the feces. Approximately 50 per cent (average) cannot be accounted for although no evidence of storage has been found.

5. Preliminary data have been obtained with intramuscularly administered Prantal. In two dogs 100 per cent recovery of the drug was obtained, the greater part being excreted in the feces.

6. By means of countercurrent distribution as well as actual isolation, the quaternary excreted in the urine has been shown to be the original Prantal quaternary moiety.

REFERENCES

(1) Perlman, P. L., and Bonsal, C., *J. Pharmacol. Exptl. Therap.*, **103**, 357(1951).
(2) Brodie, B., *et al.*, *J. Biol. Chem.*, **158**, 705(1945).
(3) Cochin, J., and Woods, L. A., *Proc. Am. Soc. Pharm. Exptl. Therap.*, Fall Meeting (1950).
(4) Zaimis, E. J., *Brit. J. Pharmacol.*, **5**, 424(1950).

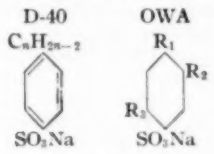
Studies on the Toxicity of Two Alkyl Aryl Sulfonates*

By CHARLES H. HINE, HAMILTON H. ANDERSON, I. FINLEY, and J. G. MOORE

Results of acute toxicity tests in rodents indicated that the two alkyl aryl sulfonates studied may be classified as only moderately toxic. Acutely poisoned animals showed evidence of stimulation and irritation of the digestive tract. The mechanism of death is obscure, since the tissue lesions were neither extensive nor pathognomonic; both compounds, however, showed more hemolytic activity *in vitro* than sodium stearate. No noticeable systemic effect followed cutaneous application, nor were there local reactions of significance. Continued feeding of the compounds to rodents resulted in no evidence of cumulative toxicity. The ingestion of small quantities would seem to be harmless.

THIS STUDY was undertaken in order to appraise the health problems which might arise from the handling of two synthetic detergents and from the ingestion of small quantities of these detergents in food stuffs.

The compounds studied were an alkyl aryl sulfonate with an average molecular weight of 352, referred to by the trade name of D-40; and a polyalkyl-aryl sulfonate with an average molecular weight of 375, referred to as OWA (Oronite Wetting Agent). The probable structural formulas of these substances are as follows:



The composition of D-40 is best represented when the *n* in the molecular formula has a value of 12. While more than one of the position isomers undoubtedly occurs, the *para* configuration is believed to be predominant. D-40 is described (1) as a detergent with a high degree of chemical stability, exceptionally uniform in its properties, of light color, and bland odor. The standard flake form is said to be about 2 per cent soluble in water at room temperature; it is more soluble in dilute ethanol, but strong ethanol solutions cause precipitation of inorganic compounds. Chemically, D-40 is a synthetic anionic detergent containing a minimum of 40 per cent sodium alkyl aryl sulfonate, approximately 2 per cent moisture, approximately 1 per cent unsulfonated oil, and the balance sodium sulfate. It is a neutral product, having a *p*H of 7.0 in a 1 per cent solution in distilled water.

OWA is a by-product of the acid treatment of oil stocks. There may be up to three alkyls on the aromatic nucleus, with a mean total of 12 to 14 carbon atoms in all substitutions. It is an anionic substance, and according to the specifications consists of sodium sulfate, 0.05 per cent; sodium sulfonate, 67.57 per cent; water by distillation, 32.0 per cent; and water insolubles, 0.01 per cent. It is described as a light-brown semifluid with a *p*H of 7.7 in 1 per cent solution in distilled water (2).

The samples of D-40 and OWA used in this evaluation were supplied by the Oronite Chemical Company. According to the manufacturer, no cases of dermatitis or systemic injury have been observed in employees engaged in their manufacture. Both agents are being used in the canning industry for the removal of dust and residual quantities of insecticide from fruits and vegetables. Chemical examination has indicated that small quantities of the detergent and wetting agent, approximately 1 p. p. m., are adsorbed on the surface of the treated products, and this circumstance made it desirable to investigate the effects of repeated ingestion.

Woodard and Calvery (3) emphasized the necessity of toxicologic investigations of the surface-active agents because of their wide field of application and their inclusion in products intended for human consumption, while deploring the variability in composition which made such studies nondefinitive.

Smyth (4) tested agents of this general group and concluded that single peroral doses are of only moderate toxicity. Benaglia (5) reported that the surface-active wetting agent he tested (the dioctyl ester of sodium sulfosuccinate) was of a low order of toxicity; periodic physical examinations of the men handling the compound daily for a period of years revealed no signs of toxicity. Klein, *et al.* (6, 7), studied the action of

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18 anionic, cationic, and un-ionized detergents on the viruses of influenza *A* and vaccinia, and on three bacteriophages. They were not active *in vivo*, though *in vitro* seven of the agents inactivated the viruses. The typical toxic manifestation in mice, after exposure to detergent sprays, was pulmonary hemorrhage. The toxicity, except for the emulsols, paralleled the activity against the virus of influenza *A*.

Ponder (8) reported some sodium salt anionic detergents of sulfonated straight-chain alcohols to be hemolytic. Reiner (9) showed that injection of anionic synthetic detergents into the tail vein of a mouse produced gangrene in twenty-four to forty-eight hours, and loss of the entire tail in a few days.

While the toxicity of synthetic detergents has not been extensively studied, considerable information is available on the toxicity and irritating properties of soap. The hemolytic properties of soaps have long been recognized. The prevailing opinion is that by virtue of their surface activity they alter the permeability of the erythrocyte membrane in such a way as to allow the hemoglobin to escape into the surrounding medium. Edwards (10) found that the hemolytic activity of soaps varied with changes in the fatty acid part of the molecule, and with changes in *p*H in a given soap. Thrombus, embolism, and pulmonary hemorrhage may result from intravenous injection of soaps.

Deaths from pulmonary embolism, septicemia, inflammatory sequelae including sterility and peritonitis, and hemorrhage, have been reported by Weilerstein (11) to result from intrauterine introduction of soap pastes.

Soaps are normally found in the intestine, and constitute about 10 per cent of the fat content of the dried feces. They usually occur in combination with sodium, calcium, or magnesium, and may be formed in the course of normal digestion. Soaps formed in this manner or ingested in small amounts do not appear to be detrimental; in fact they can be beneficial in the digestion of fats. However, it has been reported by Lewin (12) that two human deaths followed ingestion of large quantities of soap. Death might be attributed to the action of the entire soap, the cation, the anion, or the extraneous material present.

Emery and Edwards (13) suggested that soaps cause irritation by entrance into the skin, and that a minimal threshold amount must enter to irritate. It was further postulated that excess amounts would produce greater irritation, but the greatest increase in irritation would occur when the concentration was nearest to the threshold amount. On the other hand, it would seem that soaps need not penetrate the skin to cause irritation. Most soaps exert an osmotic pressure, and their ability to cause dehydration may explain the mechanism of irritation. Kooyman (14) believed that soap may act as a very mild irritant, on the basis of the universal exposure of human skin to soap with low incidence of direct irritation. The soaps of fatty acids usually act only as weak primary irritants, and not as sensitizers. Most sensitization is produced by dyes, perfumes, pigments, and preservatives added to the soap.

PERORAL STUDIES

Single Intragastric Administration

Procedure.—Five groups of albino mice of the Fisher strain (20–27 Gm. after fasting eight hours) were given 1.4 to 2.9 Gm./Kg. of D-40 intragastrically by means of a ball-point needle and syringe, and five groups were given 1.95 to 4.0 Gm./Kg. of OWA.

Four groups of Golden Syrian hamsters (90–150 Gm. after fasting fifteen hours) were similarly given 0.7 to 1.5 Gm./Kg. of D-40, and three other groups received 0.7 to 1.5 Gm./Kg. of OWA.

Three groups of immature rats of the Long-Evans strain, whose mean weight was 120 Gm. after fasting fifteen hours, were given 2.0 to 3.15 Gm./Kg. of D-40. Four similar groups were given 1.5 to 3.0 Gm./Kg. of OWA. Three groups of adult rats, whose mean weight was 200 Gm. after fasting, were given 1.1 to 2.0 Gm./Kg. of OWA.

Three groups of adult albino rabbits of the Fisher strain (2.8 to 3.5 Kg. after fasting twenty-four hours) were given 0.5 to 2.2 Gm./Kg. of D-40 intragastrically by means of an urethral catheter.

All animals were observed for the customary ten-day period.

Results.—Mortality data are presented in Table I.

Mice given D-40 exhibited a gelatinous diarrhea which contained traces of blood in 90% of the animals. A considerable decrease in motor activity of the animals was seen immediately after administration. Necropsy revealed bloody feces in the intestine, and slight hemorrhage in the pyloric mucosa. All deaths but one occurred within twelve hours. OWA produced essentially the same results.

The outstanding effects produced by both compounds in hamsters were diarrhea and decrease in motor activity. The average time of death was fourteen hours.

The rats exhibited severe diarrhea and general sluggishness. No deaths occurred before sixteen hours or later than the next day.

Rabbits exhibited the same diarrhea and sluggishness, but deaths occurred from the first until the third day.

Repeated Intragastric Administration

Procedure.—Forty healthy rats of the Long-Evans strain were divided into 4 groups of 5 males and 5 females each. The three test groups re-

TABLE I.—MORTALITY FOLLOWING SINGLE INTRAGASTRIC ADMINISTRATION OF D-40 AND OWA

Species	D-40 Dose, Gm./Kg.	D-40 Death at Hr. 2	4	Day 1	2	3	6	D-40 Mortality Ratio	D-40 LD_{50}, Gm./Kg.	OWA Wetting Agent Dose, Gm./Kg.	OWA Death at Hr. 2	4	Day 1	2	4	8	9	OWA Mortality Ratio	OWA LD_{50} Gm./Kg.
White Mice	1.4							0/10	2.01 ±	1.95			2				1	3/20	2.57 ±
	1.8			2				2/10	0.121	2.3	2		6					8/20	0.137
	2.1			6				6/10		2.8		1	5					6/20	
	2.4			8				8/10		3.4		2	6					8/10	
	2.5			9	1			10/10		4.0		3	6					9/10	
Golden	0.7							0/10	1.131 ±	0.7					1			1/10	1.0 ±
Syrian	1.0			1				1/10	0.043	1.0		1	1	1		1		4/10	0.111
Hamsters	1.2			7	1			8/11											
	1.5			8				8/8		1.5			10					10/10	
Young									2.32 ±	1.5								0/10	2.32 ±
Long-	2.0							0/12	0.122	2.0			3	1				4/15	0.122
Evans	2.6			5				5/14		2.5			1	1				2/10	
Rats	3.5			14			1	15/20		3.0			10					10/10	
Mature										1.1								0/6	1.62 ±
Long-										1.6			4					4/11	0.111
Evans										2.0			10					10/10	
Rats																			
Albino	0.5							0/4	1.73 ±										
Rabbits	1.5			1	1			2/4	0.38										
	2.2			2		1		3/4											

ceived 10, 25, and 50% of the LD_{50} (2.32 Gm./Kg.), respectively, of OWA in 10% aqueous solution. The compound was administered daily for thirty days.

The rats were weighed weekly, growth curves were computed, and at the end of thirty days the survivors were decapitated and examined for gross lesions.

Results.—One of the young male rats given 25% of the LD_{50} died for unexplained reasons on the thirteenth day; its weight gain had been normal. No other deaths occurred, nor were significant changes found in any of the animals on necropsy. There was, however, a definite inhibition of growth in the groups receiving half the LD_{50}. Inhibition was questionable in females receiving 25% of the LD_{50}.

Forty-Five-Day Feeding

Procedure.—Forty healthy young adult rats of the Long-Evans strain were divided into groups as before. These were inbred rats from 10 litters, weighing at the beginning of the experiment 150 ± 15 Gm. The first group served as control and was fed standard dog chow. The other groups received 10, 25, or 50 per cent of the LD_{50} of D-40 in each 16.6 Gm. of stock diet (the average daily intake).

The food was weighed and replenished every other day, and was dispensed from special metabolism cups which prevented spilling and permitted accurate weighing of the quantity consumed. The rats were observed daily for changes in activity and appearance, and were weighed weekly for the forty-five days of the experiment. They were then killed and examined for pathologic changes.

Results.—The average daily food consumption and weight change in the rats fed D-40 were equal to or greater than those of the controls, indicating that the daily ingestion of these relatively large amounts of detergent had no adverse effect (Table II). The average weight gain of the male controls was 40.2 Gm.; that of the females was 35.6 Gm. No pathologic changes were seen at necropsy.

TABLE II.—MEAN DAILY FOOD CONSUMPTION AND WEIGHT CHANGE IN RATS FED D-40 FOR FORTY-FIVE DAYS

% LD_{50} in Diet	Sex	Food Intake, Gm.	Weight Change, % of Control
0	M	16.9	...
(Control)	F	14.2	...
10	M	18.3	+136
	F	16.0	−17
25	M	18.3	+157
	F	14.7	+26
50	M	18.2	+78
	F	15.2	+22

Six-Month Feeding

Procedure.—Chronic toxicity studies were carried out on groups of rats of the Long-Evans strain (85 ± 15 Gm.) obtained from the inbred stock of the Institute of Experimental Biology of the University of California at Berkeley. After six months on a diet containing D-40 or OWA, results were evaluated on the basis of mortality; retardation of growth; abnormal organ/body weight ratios; clinical laboratory evidence of renal and bone-marrow damage; and gross and microscopic alterations in tissue structure.

Twenty male and 60 female rats, segregated according to sex, were maintained in groups of 5 in numbered cages. Their diet consisted of finely ground standard animal chow, supplemented weekly with fresh greens. The detergent or wetting agent was intimately mixed with the feed, at levels of 1, 10, and 20 p. p. m. Ten animals were maintained at each dose level of each compound, and 20 were left as controls. The feed was constantly available in metabolism jars with mesh tops, which were weighed and refilled every three days. The average consumption of feed was recorded for each group; water was allowed *ad libitum*.

The individual rats were removed from their cages weekly, carefully inspected, their activity

noted, and their weight recorded. Once during the fifth month, and again during the sixth month, each animal was placed in a metabolism cage for twenty-four hours for urine collection. The samples were analyzed chemically for albumin (sulfosalicylic acid method) and for sugar (qualitative Benedict test), and microscopically for renal casts, leukocytes, and erythrocytes. Blood studies, including hemoglobin determination, red and white cell count, and differential count, were performed at the time of necropsy.

At the end of the sixth month, all animals were killed by anesthetization with ethyl ether, without exsanguination. A necropsy was performed on each rat, and the weight of liver, kidneys, heart, lung, spleen, and testes was determined. The organs were blotted before being weighed. Appropriate sections of liver, kidney, spleen, heart, lung, stomach, intestine, and brain were removed and fixed in 10% formalin, and stained with hematoxylin-eosin for histologic examination. The data were analyzed by customary statistical techniques.

Results.—Throughout the experimental period, the rats appeared healthy and normally active, except for one rat on a diet of 1 p. p. m. D-40, which died during the third week. Its death was not attributable to the test diet.

There was a significant difference in food consumption between male and female rats. The only significant difference between a test group and a control concerned the females receiving 20 p. p. m. of OWA; the monthly food consumption of this group increased. Both deviations were at the 0.05 level. Consumption of both groups was, however, within normal limits, and it was concluded that the presence of D-40 and OWA did not adversely affect food intake.

There were no significant differences in weight gain, which indicated that neither D-40 nor OWA affected growth adversely.

Clinical Laboratory Tests.—Unfiltered urine specimens from both test and control groups gave slightly positive tests for albumin and reducing substances, but the results were negative after the urine had been passed through filter paper.

Centrifuged specimens of unfiltered urine from normal animals showed from 0 to 5 leukocytes and 0 to 7 erythrocytes per high-power field, when examined microscopically. These values are therefore considered to lie within the normal range. Urine from the test animals was similar, and it was concluded that no renal damage detectable by these methods had occurred.

The hemoglobin values and red cell counts were within normal limits in all groups. There was no evidence of increased red or white cell destruction or of abnormal cell distribution.

Organ Body Weight Ratios.—A few statistically significant values were obtained when the organ/body weight ratios were compared (Table III). The kidneys of females receiving 20 p. p. m. of D-40 and of males receiving 20 p. p. m. of OWA were heavier than those of the controls ($p = 0.01$). The cause is not apparent; there was no evidence of kidney damage by clinical analysis of the urine or microscopic examination of tissue sections. In four instances, the lungs of the control group females were heavier than those of females in the D-40 group. The reason for the difference is uncertain. Some animals, especially among the female controls, showed a nonspecific fibrosis on microscopic examination, but it was not possible to estimate the total fibrosis of the lung from the sections available. It is not suggested that the fibrosis would account for the increase in lung weight *per se*, but it undoubtedly affected the drainage of blood at necropsy. Increased retention of blood may account for the increased weight among the controls. The difference in the spleen ratios is not unusual, since there are often differences due to spleen volume in anesthetized animals. There was neither gross nor microscopic evidence of spleen damage, though the size of the organ varied considerably. Two females on 20 p. p. m. of OWA had spleens which were 60 and 70% larger than those of the controls.

Lesions.—All tissues were inspected *in situ* within thirty minutes after death, and there was no gross evidence of abnormality except in one rat. This was a female on 20 p. p. m. OWA, which was observed to have several apparently benign hepatomas. There was no evidence of parasitism, and the rats appeared to be in excellent condition. The mucosa of the stomach, small intestine, and colon was closely inspected, but no evidence of irritation was found. All organs appeared slightly engorged with blood, as is to be expected following death by etherization.

Sections of lung, heart, spleen, liver, pancreas, kidney and brain were examined microscopically by Dr. W. Bostick of the Pathology Department. His findings were negative, e. g.: "Liver: The cells are well preserved showing no fat infiltration

TABLE III.—STATISTICAL DIFFERENCES IN ORGAN/BODY WEIGHT RATIOS ENCOUNTERED IN THE CHRONIC FEEDING EXPERIMENT

Compound	Sex	Statistical Difference in Organ/Body Weight Ratios		
		Kidney	Lung	Spleen
D-40	F	....	1 p. p. m. (0.05)	1 p. p. m. (0.01)
		....	Control 7% heavier	Control 20% heavier
		10 p. p. m. (0.01)	10 p. p. m. (0.05)	10 p. p. m. (0.01)
		Control 8% lighter	Control 20% heavier	Control 10% heavier
		20 p. p. m. (0.01)	20 p. p. m. (0.05)	20 p. p. m. (0.01)
		Control 8% lighter	Control 20% heavier	Control 20% lighter
OWA	F	....	1 p. p. m. (0.01)	20 p. p. m. (0.001)
		....	Control 8% heavier	Control heavier
		....	10 p. p. m. (0.01)	....
		....	Control 16% lighter	....
	M	20 p. p. m. (0.01)	....	....
		Control 16% lighter	....	....

and in one instance a slight degree of fibrosis which is probably old and not related to the experiment. Kidney: The glomeruli are delicate and the tubules throughout show no significant changes, no necrosis, and no inflammatory infiltration. Brain: The neuron cells are well preserved. There is some scattering of congestion in the meninges, but no perivascular infiltration or evidence of specific degeneration. Conclusion: No morphologic lesions caused by agents administered."

CUTANEOUS TOXICITY

The possibility of percutaneous absorption of D-40 and OWA in quantities sufficient to cause a systemic effect was evaluated, in accordance with the methods of Draize, *et al.* (15), by the application of measured amounts of the substances to the depilated skin of adult albino rabbits. Twenty hours were allowed to elapse after the rabbits were shaved, to permit healing of any accidental abrasions before the test.

D-40 or OWA was applied undiluted, in amounts equivalent to the intragastric LD_{50}, and 0.24 and 1.75 times the logarithm of that dose. The rabbits were immobilized in a multiple rabbit holder, as described by Laug (16). The test area was covered with gauze, and the animal's trunk wrapped in a rubber sleeve. After the exposure period of twenty-four hours, the unabsorbed portion of the compound was washed off, and the condition of the skin was recorded. Further examinations of the skin were made at forty-eight and ninety-six hours after the application. The rabbits were observed over a period of ten days, for signs of physiologic disturbance.

There were no deaths, and no evidence suggesting significant absorption of either D-40 or OWA. There was moderate edema, erythema, and scabbing of the skin at the site of application, but the skin of all animals returned to normal and the damage was not severe.

This single-exposure was followed by an experiment in which either D-40 or OWA was applied repeatedly to the skin of rats (240–420 Gm.) and rabbits (2.4–2.65 Kg.). Applications were made daily except Sunday, for thirty days. Five per cent aqueous solutions were applied with the aid of a syringe and a glass rod, in doses equal to 0.07 Gm./Kg. for the two groups of 6 rats, and 0.02 Gm./Kg. for the two groups of 3 rabbits.

None of the animals showed any evidence of toxicity following the series of exposures, and the rats showed no signs of skin irritation. One rabbit showed a +1 erythema, lasting one day, on the eleventh day of D-40 application, and one rabbit treated with OWA showed a +1 erythema on the twenty-eighth day, which persisted for the remaining two days of the experiment.

IRRITATION AND SENSITIZATION

Skin Irritation.—The testing and scoring of skin irritating effects of the detergent and the wetting agent were also carried out according to the procedure of Draize, *et al.* (15). Albino rabbits, prepared by shaving twenty hours previously, were immobilized in the multiple holder. Four areas on each rabbit, approximately 2.5 $cm.^2$ and 10 cm. apart, were selected as test sites; two of these were abraded with fine mesh wire. One-half gram of the undiluted compound was placed on each site, held in place by a thin square of gauze and adhesive tape. The animal's trunk was then wrapped in a rubber sleeve. After an exposure period of twenty-four hours, the remaining test material was removed, and the amount of skin irritation was evaluated. A second reading was made at seventy-two hours.

Barely perceptible irritation is scored by Draize as +1 erythema, and the score increases numerically to +4 at beet redness or depth injury. Edema is scored similarly, with swelling greater than 1 mm. in height and extending beyond the exposure area indicated as +4.

No readings were obtainable on the unabraded skin of the rabbits to which D-40 was applied. All the others showed slight, persistent edema and erythema. The results are shown in Table IV.

Eye Irritation.—An evaluation of the eye-irritating effect of D-40 and OWA was made, according to the procedure of Draize, *et al.* (15). One-tenth ml. of a 5% aqueous solution was placed on the cornea of one eye of normal rabbits, the other eye serving as a control. The eyes were preselected on the basis of absence of gross visible staining by 5% aqueous fluorescein sodium, flushed with distilled water twenty seconds after instillation. Two hours later, after the eyes had returned to normal, the test compounds were introduced.

TABLE IV.—PRIMARY SKIN AND EYE IRRITATION[a] OF D-40 AND OWA FOR ALBINO RABBITS

Compound	Treatment	Skin Condition at 24 Hours Erythema	Skin Condition at 24 Hours Edema	Skin Condition at 72 Hours Erythema	Skin Condition at 72 Hours Edema	Eye Condition at 1 Hr.	Eye Condition at 24 Hr.	Eye Condition at 96 Hr.	Eye Total Score	Average Score
D-40	Unabraded	−	−	−	−	..	..	..	..	
		−	−	−	−	..	..	..	..	
		−	−	−	−	8	2	0	10	10
	Abraded	+1	+1	+1	+1	8	2	0	10	
		+1	+1	+2	+1	..	..	..	..	
		0	+1	0	+1	..	..	..	..	
OWA	Unabraded	+1	+1	+1	+1	..	..	..	..	
		+1	+1	+1	+1	..	..	..	..	
		+2	+1	+2	+1	4	11	0	15	11.5
	Abraded	+1	+1	+2	+1	6	2	0	8	
		+1	+1	+2	+1	..	..	..	..	
		+1	+1	+2	+1	..	..	..	..	

[a] Scored according to the method of Draize, *et al.* (15).

Observations were made at one, twenty-four, and ninety-six hours. A moderate response was provoked by both substances, but all visible signs of irritation had disappeared by the time of the final reading. There was no loss of corneal substance, as indicated by the fluorescein test for break in continuity of the epithelium. Table IV gives the mean numbered scores at the various time intervals.

Sensitization.—The sensitizing ability of D-40 and OWA was tested on groups of 3 albino guinea pigs, according to the method of Landsteiner and Jacobs (17). A 0.1% solution was made of each compound in 0.9% sodium chloride and injected intradermally on alternate days in an area of approximately 4 cm.², below the midline of the back. The first injection was 0.05 ml. and subsequent doses were 0.01 ml. Readings were made after twenty-four hours. Two weeks after the tenth and last sensitizing dose, a test injection of 0.05 ml. was made in the flank.

Twenty-four hours after the challenging injection, no erythema or wheal formation was discernible at the test site in any animal. This would suggest a lack of sensitizing ability in the two compounds.

MECHANISM OF ACTION

The action of these sulfonates *in vivo* has suggested that a possible mechanism of action lies in their ability to produce hemolysis and thrombosis. An estimation was therefore made of the hemolytic activity of D-40 and OWA *in vitro*. Pure sodium stearate and commercial soap flakes were tested at the same time for comparative purposes.

The procedure was adapted from that of Medveczky and Votin (18). Dilutions of the test substances ranging from 1:1,250 to 1:10,000 were prepared in distilled water. One-half ml. of each solution was adjusted to *p*H 7.0 with 0.4 ml. of isotonic phosphate buffer (0.154 *M*). Similar solutions were buffered to *p*H 7.35. To these was added 0.6 ml. of physiological saline solution, and 0.5 ml. of 0.5% suspension of washed red blood cells of sheep. Control tubes contained only red cells and saline at each *p*H. The tubes were agitated for three minutes, and then allowed to stand at room temperature for thirty minutes. The degree of hemolysis was scored as follows:

0 = no hemolysis
+ = detectable hemolysis
++ = moderate hemolysis
+++ = nearly complete hemolysis
++++ = complete hemolysis

It can be seen from the data presented in Table V that both D-40 and OWA had a slightly greater hemolyzing power than the soap flakes, and about 100 times the hemolyzing power of pure sodium stearate. It was also observed that the rate of hemolysis caused by the higher concentrations of OWA was very rapid, being a matter of only a few seconds, while that caused by soap solutions was more slowly. Change of *p*H from 7.0 to 7.35 did not result in significant differences. The lower limit of hemolyzing concentrations was 1:7,500 for D-40 and 1:10,000 for OWA.

DISCUSSION

Acute toxicity tests did not demonstrate any appreciable difference in species susceptibility to D-40 or OWA in the four rodent species tested. The LD_{50} values, ranging from 1.0 Gm./Kg. in the hamster to 2.77 Gm./Kg. in the young rat, indicated that these alkyl-aryl sulfonates may be classified as only moderately toxic compounds. The acutely poisoned animals did not display any remarkable signs of altered physiology. The gelatinous feces with traces of blood, which frequently followed intragastric administration, were evidence of stimulation and irritation of the gastroenteric tract. However, gross and microscopic examination of the bowel resulted in no remarkable findings. Petechial hemorrhages and thrombosis were the only evidences of pathologic change.

The mechanism producing death is obscure, since the tissue lesions were neither extensive nor pathognomonic. However, the studies *in vitro* show that D-40 and OWA have considerably more hemolytic activity than sodium stearate, and this fact may have some bearing on the problem.

The studies on cutaneous toxicity demonstrated that neither compound was absorbed through the skin in quantities sufficient to cause a noticeable systemic effect. Irritation studies also showed the substances to be relatively innocuous on single or repeated contact. However, although there would not appear to be any likelihood of either systemic or local reactions of any significance following exposure to these compounds, an occasional case of dermatitis may be expected to

TABLE V.—THE IN VITRO HEMOLYZING PROPERTIES OF D-40 AND OWA COMPARED WITH SOAP AND SODIUM STEARATE

Compound	*p*H	Control	Dilutions							
			1:1,250	1:1,500	1:1,750	1:2,000	1:4,000	1:6,000	1:8,000	1:10,000
Soap flakes	7.0	0	++++	++++	++++	++++	+	0	0	0
	7.35	0	++++	++++	++++	++++	+	+	+	0
							1:2,500	1:5,000	1:7,500	
D-40	7.0	0	++++	++++	++++	++++	++++	++	0	0
	7.35	0	++++	++++	++++	++++	++++	++	0	0
OWA	7.0	0	++++	++++	++++	++++	++++	++	+++	+
	7.35	0	++++	++++	++++	++++	++++	++	+	0
			1:20	1:25	1:33	1:50	1:125	1:150	1:175	1:200
Sodium stearate	7.0	0	++	++	+	0	0	0	0	0
	7.35	0	...	...	+++	++	0	0	0	0

occur. Lane and Blank (18) demonstrated that long exposure to concentrated soap solution would almost invariably result in irritation, although few persons showed reactions to a one or two per cent soap solution. Hypersensitiveness was cited as the usual explanation for dermatitis accompanying daily use of cleansing agents; it is more likely to appear in previously sensitized individuals.

The subacute and chronic feeding studies in rodents failed to incriminate the sulfonates as cumulatively toxic. Based on the factors of absence of significant tissue changes, normal clinical laboratory findings, normal weight gains, and essentially normal organ/body weight ratios, our belief is that the ingestion of small quantities of the alkyl aryl sulfonates would be without harmful effects in man.

The relatively large quantities of the detergent necessary to produce toxic effects, and the absence of irritating properties, suggest that the hazard in handling these agents is negligible.

SUMMARY

1. The peroral toxicity of two alkyl aryl sulfonates, D-40 and OWA, was evaluated by single intragastric administration to mice, rats, hamster, and rabbits; by repeated intragastric administration to rats; and by the feeding of the compounds to groups of rats for periods of forty-five days and six months.

2. The question of percutaneous absorption and consequent systemic reaction was decided by single applications to the skin of rabbits, and repeated applications to both rabbits and rats.

3. Irritation and sensitization studies were carried out on rabbits and guinea pigs, and the hemolytic activity of the sulfonates was compared with that of sodium stearate and soap flakes *in vitro*.

4. No significant problems in handling the compounds are anticipated, nor is the ingestion of small quantities believed to be hazardous, according to the results of these studies.

REFERENCES

(1) Oronite Chemical Company, Tech. Bull. (1945).
(2) Oronite Chemical Company, Tech. Bull. (1944).
(3) Woodward, G., and Calvery, H. O., *Proc. Sci. Sect. Toilet Goods Assoc.*, **3**, 1(1945).
(4) Smyth, H. F., Seaton, J., and Fisher, L., *J. Ind. Hyg. Toxicol.*, **23**, 478(1941).
(5) Benaglia, A. E., Robinson, E. J., Utley, E., and Cleverdon, M. A., *ibid.*, **25**, 175(1943).
(6) Klein, M., Kalter, S. S., and Mudd, S., *J. Immunol.*, **51**, 389(1945).
(7) Klein, M., and Stevens, D. A., *ibid.*, **50**, 265(1945).
(8) Ponder, E., *J. Gen. Physiol.*, **30**, 15(1946).
(9) Reiner, L., *Proc. Soc. Exptl. Biol. Med.*, **62**, 49 (1946).
(10) Edwards, L. D., THIS JOURNAL, **28**, 209(1939)
(11) Weilerstein, R. W., *J. Am. Med. Assoc.*, **125**, 205 (1944).
(12) Lewin, L., "Gifte und Vergiftung," G. Stilke, Berlin, 1929.
(13) Emery, B. E., and Edwards, L. D., THIS JOURNAL, **29**, 254(1940).
(14) Kooyman, D. J., and Snyder, F. H., *Arch. Dermat. and Syphilol.*, **46**, 846(1942).
(15) Draize, J. H., Woodward, G., and Calvery, H. O., *J. Pharmacol. Exptl. Therap.*, **82**, 377(1944).
(16) Laug, E. P., *J. Lab. Clin. Med.*, **29**, 308(1944).
(17) Landsteiner, K., and Jacobs, J., *J. Expt. Med.*, **61**, 643(1935).
(18) Lane, C. G., and Blank, I. H., *J. Am. Med. Assoc.*, **118**, 804(1942).

Anticonvulsant and Growth Inhibitory Effects of Some Organophosphorus Compounds*

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A series of organophosphates, structurally phosphoryl analogs of phenylurethane, have been tested for anticonvulsant and growth inhibitory effects. The effects were expected from structural considerations.

THERE ARE a large number of biologically important compounds that may be pictured as being built around the two chemical groups C:O and P:O. Our studies (1) on the effect of substituting the P:O group for the C:O group in phenylurethane yielded results that were in favor of the assumption that the phosphoryl analogs of phenylurethane might mimic its biological effects. We have not found in the literature any report of an anticonvulsant effect by an organophosphate. This report is therefore of sufficient theoretical interest.

The synthesis of, and the physical data on, the compounds are published elsewhere (2).

EXPERIMENTAL

Pharmacological Effects of the Compounds.—The anilidophosphates were tested for the following effects: (*a*) toxicity, and (*b*) anticonvulsant action measured in terms of protection against electroshock and against metrazol injected intravenously.

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† Experimental data taken in part from a dissertation submitted by Dasu Ramaswami in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Chemistry.
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Harlan strain mice of weight varying from 16 to 21 Gm. were used.

The water-insoluble members of the series were made up as suspensions in distilled water using 200 mg. of finely powdered tragacanth as the suspending agent per 20 ml. of the solution. The water-soluble ones were dissolved in distilled water.

They were administered intraperitoneally; orally they did not show much measurable effect.

Toxicity.—Preliminary tests showed that the series had a mild effect only. Therefore, the routine procedure of determining the LD_{50} was not followed. Instead, the lethal dose on three mice was considered as an index of the toxicity of the compound. Of three graded doses the smallest that killed the animal overnight was considered the lethal dose.

The following toxic effects were observed. All the compounds produced abdominal irritation, spasticity, excitement, general depression, and hyperpnea. A slight degree of analgesia in regard to ear prick, foot pinch, and tail pinch was noticed. The mice all returned to normal in two to three hours.

One hour after the administration of the compound, the animal was tested for protection against electroshock using Lampier 8-47 sine wave stimulator passing the current for an interval of three-tenth of a second.

The relatively low potency of the series suggested the use of a current strength of 15 ma. at which a normal mouse goes into convulsions. At higher toxic dose levels, however, the compounds offered protection against a current as high as 50 ma. in some cases.

Similarly another series of treated animals was tested for protection against Metrazol. Metrazol was administered intravenously until the animal had a convulsive seizure. Controls were run on untreated mice and the difference in dose of Metrazol inducing a convulsion in treated and untreated mice was considered as a measure of protection offered by the compound. None of the compounds offered any protection against Metrazol. Results of the systematic testing of the compounds are shown in Table I.

TABLE I.—TOXIC EFFECT OF COMPOUNDS TESTED

Compound	M. T. D.,[a] Mg./Kg.	Anticonvulsant Effect M. E. D.,[b] Mg./Kg.	L. D.,[c] Mg./Kg.
Phenylurethane	100	150	400
Diethyl anilidophosphate	100	533	666
Di-*n*-propyl anilidophosphate	125	300	500
Diisopropyl anilidophosphate	300	900	1,500
Di-*n*-butyl anilidophosphate	100	300	400
Diisobutyl anilidophosphate	500	1,000	2,000
Di-*n*-amyl anilidophosphate	500	500	750
Diisoamyl anilidophosphate	1,000	1,500	2,000
Diethyl anilido-thiophosphate	100	333[d]	500
Benzene ethyl anilidophosphonate	500	1,000	2,000
Diphenyl anilidophosphate	1,000	2,000	2,500

[a] M. T. D., minimal toxic dose, at which dose mild toxic signs were noticed.
[b] M. E. D., minimal effective dose, at which dose the mouse was protected against a current of 15 ma.
[c] L. D., lethal dose which killed one mouse; this is not LD_{50}.
[d] Produced convulsions.

Differential Effect of the Anilidophosphates upon Plant Species.—The effects of phenylurethane upon some monocotolydenous plants, wheat, and oat seedlings have been described by Lefèvre (3). Morphologically, they resemble those observed with colchicine, *viz.*, the bulbous hypertrophy of the coleoptile, the mesocotyl, and the root tip, with progressive slowing down or even final arrest of growth. The same effects have been observed in varying degree of intensity in the cases of chloral hydrate, and isopropyl carbamate. These compounds are without effect on seeds of yellow charlock (*Brassica sinapis visiani*), a dicotyledonous plant (4). It is tempting to assume the possibility of a relationship between this inhibitory effect and the hypnotic effect possessed by these same compounds. However, Templeman and Sexton did not find any such significant relationship when they studied other known hypnotics (4).

From structural considerations a similar differential effect upon plant species was predicted for the present series of anilidophosphates.

The procedure was (with some modifications) that of Templeman and Sexton (5). Fifteen to twenty ml. of the solution was placed in a watch glass of 5 in. diameter. A filter paper (diameter 12.5 cm.) was spread on the watch glass, the edge of the paper coming flush with the rim of the watch glass. The paper was pressed down gently with the forefinger to touch the solution in the watch glass and the moist area allowed to spread throughout the paper. Selected seeds (on the basis of physical appearance) were placed on the moist paper all around and within one-half cm. from the rim of the watch glass. This arrangement affords a convenient means of providing a moist paper surface upon which the seeds can be placed without letting them be immersed in the solution. Another watch glass of the same size was laid over this set-up with the concave sides facing.

The solutions were made by dissolving 0.1 m*M* of the compound in 100 ml. of distilled water giving a concentration of one mole in a million ml. of the solution. Dibutyl and diamyl anilidophosphates, both normal and iso, which are insoluble, were treated in the following way. The weighed quantity of the compound was dissolved in $^1/_2$ ml. of ethanol and 99.5 ml. of distilled water quickly added to the flask which was shaken vigorously.

Each solution was tested on selected seeds of oat (*Avena sativa* L.) and yellow charlock (*Brassica sinapis visiani*), fifty seeds in each test, running controls with (*a*) distilled water and with (*b*) phenyl urethane solution of the same concentration all at the same time. The observation lasted five days. The results are shown in Table II.

The compounds under investigation produced one or more of the following effects: (*a*) Inhibition of root growth: 70% of the seeds showed a root growth less than 5 mm. long, the roots appearing brown and rigid in contrast with those in the control using distilled water which were white, hydrated, and 9 cm. long. (*b*) Inhibition of shoot growth: over 70% of the seeds showed a shoot growth of 5 mm. (*c*) Slight retardation of shoot and root growth: roots

and shoots grew to 75% of normal length. (*d*) Severe retardation of shoot growth: shoot grew to less than 50% of the length of the normal. (*e*) Severe retardation of root growth: roots grew to a third of normal length.

In the case of yellow charlock seeds the percentage of germination was the same for the controls (with distilled water) as well as in the case of the anilidophosphates, i. e., less than 50%.

TABLE II. INHIBITING EFFECT OF COMPOUNDS TESTED

Compound	Effect on Germination: Oats	Charlock
Phenyl urethane	4+[a]	No effect
Diethyl anilidophosphate	1+	No effect
Di-*n*-propyl anilidophosphate	3+	No effect
Diisopropyl anilidophosphate	2+	No effect
Di-*n*-butyl anilidophosphate	3+	No effect
Diisobutyl anilidophosphate	2+	No effect
Di-*n*-amyl anilidophosphate	2+	No effect
Diisoamyl anilidophosphate	2+	No effect

[a] 1+, slight retardation in growth of roots and shoot.
2+, root growth severaly retarded and shoot growth slightly.
3+, root growth inhibited and shoot growth severely retarded.
4+, root and shoot growth inhibited.

DISCUSSION

It is an interesting observation that the dialkyl anilidophosphates, like the carbamates of which they are the analogs, affect monocotyledonous seeds only. However, no compound of the series has shown a potency equal to that of phenylurethane on oat seeds. Di-*n*-propyl and di-*n*-butyl anilidophosphates, the most potent of the series, do not completely inhibit the growth of the shoot. The exact replication of the pattern of the biological effects is, nevertheless, significant.

The changes in biological effects observed with increasing numbers of carbon atoms in this homologous series are in conformity with the prevailing ideas on that aspect. An increase in the activity is observed as the series is ascended, the peak being reached in the di-*n*-butyl member. The next higher member and its isomer are much less potent. The decrease in potency in this instance may be due to the increased insolubility of the members. Branching in the alkoxy radical reduces potency and toxicity.

Considering the effect of changes in the nucleus, substitution of oxygen of the P:O group in diethyl anilidophosphate with sulfur results in the loss of anticonvulsant effect. In fact, the diethyl anilidothiophosphate shows a mild convulsant effect. In ethyl benzene anilidophosphonate, the benzene radical substituting for an ethoxy in diethyl anilidophosphate, does not obliterate the anticonvulsant effect. This is to be expected. This compound still fulfills the requirements of the exact analog of phenylurethane since it has the basic structure $P(O)(OC_2H_5)(NH.C_6H_5)$. The molecule is in constant motion and part of the time it will assume a position in space completely analogous to that of phenylurethane $C(O)(OC_2H_5)(NH.C_6H_5)$.

Replacement of alkyl groups by phenyl groups as in diphenyl anilidophosphate still exhibits an anticonvulsant effect, though the effect is feeble at nontoxic concentrations; at the same time toxicity is reduced. At toxic levels, the compound raises the electroshock threshold of the animal considerably.

The effect of branching in the series is in interesting contrast with that in the carbamates where branching results in increased activity.

The anticonvulsant effects of the series show an exact parallelism with the growth inhibitory effects, increasing in potency with increase in the number of carbon atoms of alkoxy groups up to four and then noticeably decreasing.

We believe that the regularity with which the present series manifests the specific biological activity cannot be due to a fortuitous coincidence but is due to their structural pattern with the $-PONH-$group as the nucleus. Our results show a broad agreement with the postulated hypothesis in regard to the anticonvulsant effect and less strikingly the selective growth inhibitory effect. However, neither phenylurethane nor its analogs compare well in their anticonvulsant effect with phenobarbital sodium, a well-known anticonvulsant, structurally unrelated to the present series. It is interesting that the dipropyl and the dibutyl esters are the most potent of the series, both in inhibiting germination and in anticonvulsant activity; it would be of interest to find out if they mimic the third biological effect, *viz.*, the retardation of experimental tumors. It has been recently shown that *N*-substituted phosphoramides exert this effect (6). This possibility is under investigation.

SUMMARY

1. The hypothesis that phosphorus analogs of phenylurethane should mimic the biological effects of the latter was tested.

2. A series of dialkylanilidophosphates were tested for (*a*) anticonvulsant action on mice, measured in terms of protection against electroshock and Metrazol, and for (*b*) their selective inhibitory effect on the germination of oat and yellow charlock seeds. In both cases, their activity was compared with that of phenylurethane.

3. In the series, di-*n*-propyl and di-*n*-butylanilidophosphates were the most potent and the latter the most toxic.

4. The series showed some effect in retarding the growth of the roots and the shoot of oats. They did not show any effect on the seeds of yellow charlock.

REFERENCES

(1) Ramaswami, D., Kirch, E. R., and Jenney, E. H., *Science*, **116**, 58(1952).
(2) Ramaswami, D., and Kirch, E. R., *J. Am. Chem. Soc.*, **75**, 1763(1953).
(3) Lefèvre, J., *Compt. rend.*, **208**, 301(1939).
(4) Templeman, W. G., and Sexton, W. A., *Proc. Roy. Soc. London*, **B133**, 480(1946).
(5) Templeman, W. G., and Sexton, W. A., *ibid.*, **B133**, 300(1946).
(6) Burchenal, J. H., Goetchius, S. K., Stock, C. C., and Hitchings, G. H., *Cancer Research*, **12**, 251(1952).

A Study of Suppository Bases. III. A New Water-Soluble Suppository Base*

By HERBERT MICHEAL GROSS† and CHARLES H. BECKER‡

A new water-soluble suppository base with a high melting-point range and a high percentage medicinal release has been developed from polyoxyethylene 30 stearate. This new base was investigated for compatibility, toxicity, and microbial growth support. Polyoxyethylene 30 stearate was mixed with varying percentages of white wax depending upon the nature of the included medicament so as to result in a product with a melting point of 50°. Small quantities of Aerosol OT and water were added to the base. The mixture of polyoxyethylene 30 stearate, water, wax, and Aerosol gave excellent results in the tests conducted and is recommended as a suppository base.

THE FIRST paper in this series, a historical review (1), indicated that many formulas for suppository bases have been suggested, tried, and used. The objective was to produce a suppository of suitable characteristics for molding, shipping, and storage, but of a nature that would allow ready release of the incorporated medicament. In a second paper (2) some previously reported and some new suppository bases were evaluated by a colorimetric method. The results of this project suggested new bases for further study.

In this investigation, these bases were modified and tested for their percentage medicinal release, compatibility, storage capacity, melting-point range, and toxicity. Efforts were directed toward producing a suppository base of a high melting point, around 50°, having a ready medicinal release.

EXPERIMENTAL

Preparation of Suppositories.—All suppositories considered in this investigation were prepared by the fusion process. Medicinals were dissolved or thoroughly incorporated into the bases and poured into chilled molds when the base showed tendency to solidify.

Materials Used.—Drugs and chemicals used were of U. S. P. grade wherever possible. Reagents were of analytical grade. Unofficial products and commercial specialties were of food and drug law quality.

Test for Percentage Medicinal Release.—The colorimetric method described in a previous article (2) was used.

Melting-Point Determination.—The melting-point range was determined by the method outlined in U. S. P. XIV (3), following the directions for materials of Class II.

Suppository Disintegrating Agents.—Two disintegrating agents, ground sponge and citrus pulp (4), were tested as modifiers of suppository bases.

Modified Carbowax and Polyoxyethylene Stearate Bases.—Carbowax 1000 and Carbowax 4000 monostearates and polyoxyethylene 30 and polyoxyethylene 40 stearates were modified with the following substances: water, triethylene glycol, sorbitol, white wax, cetyl alcohol, Aerosol OT, citrus pulp, sponge, and combinations of these. The modifications were tested for their percentage medicinal release and were compared with the unmodified base. The results are presented in Table I. In addition, some of the best modifications appearing in Table I were compared with one another for percentage medicinal release from the base and with theobroma oil. Comparative data of these combinations are shown in Table II.

Microbiological Testing.—Carbowax and polyoxyethylene stearate bases in combination with water were tested to determine if they would support microbial growth. Since included water could enhance the possibility of microbial growth, 10% water was added to each base and testing was performed on these products. Gram-positive and Gram-negative bacteria, molds, and yeasts were added to the bases. Some plates were exposed open to the air. Anaerobic and aerobic tests were performed. The organisms used were: *E. coli*, *B. subtilis*, *A. niger*, *Mucor*, and *S. cerevisiae*. At interval times, the plates and tubes were examined for growth, slides prepared, and at the end of two weeks' incubation at 37° data were compiled. The results of the bacteriological tests carried out showed that the tested bases apparently do not support microbial growth.

A New Water-Soluble Suppository Base.—Of the various modifications made with Carbowax and polyoxyethylene stearate bases, the best suppository base from the standpoint of percentage medicinal release, melting-point range, and physical appearance was polyoxyethylene 30 stearate containing white wax, water, and Aerosol OT. The physical properties and commercial source of polyoxyethylene 30 stearate are given in a publication by the Atlas Powder Company (5).

Compatibility Tests.—General spot compatibility tests were performed on this base to check its pharmaceutical feasibility. Medicinals frequently found in suppositories were tested for compatibility. Percentages corresponding to doses of medicinals commonly used in suppositories were incorporated into the proposed suppository base. The water-soluble medicinals were first dissolved in a small amount of water before incorporation into

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TABLE I.—THE EFFECT ON PERCENTAGE MEDICINAL RELEASE BY MODIFICATION OF SOME CARBOWAX AND POLYOXYETHYLENE STEARATE BASES IN A NINE-MINUTE TEST

	Percentage Dye Released from Modified Base as Compared to Unmodified Base												
	Base Modifiers												
Original Base	100% Base	10% Water	20% Water	10% Triethylene Glycol	10% Sorbitol	5% White Wax	5% Cetyl Alcohol	1% Aerosol OT	3% White Wax, 5% Water, and 0.5% Aerosol OT	5% White Wax, 10% Water, and 0.5% Aerosol OT	1% White Wax, 10% Water, and 0.5% Aerosol OT	10% Citrus Pulp	1% Sponge
Carbowax 1000 monostearate[a]	100.0	52.5	...	...	...	51.8	80.0	...	...	24.4	...	...	...
Melting-point range, ° C.	42–44	38–40	...	...	...	49–52	37–39	...	...	47–50	...	...	...
Carbowax 4000 monostearate[a]	100.0	208.0	211.0	197.0	84.5	...	...	207.0	...	...	234.0	177.0	192.0
Melting-point range, ° C.	52–54	47–49	40–42	44–47	56–60	...	...	48–52	...	...	52–54	55–58	52–56
Polyoxyethylene 30 stearate[b]	100.0	181.0	...	...	...	177.0	66.2	73.5	181.0	...	...	...	...
Melting-point range, ° C.	42–46	40–43	...	...	...	52–55	40–43	39–41	49–51	...	...	...	...
Polyoxyethylene 40 stearate[b]	100.0	225.0	...	172.0	58.5	17.8	55.7	...	...	203.0	...	178.0	198.0
Melting-point range °C.	44–46	42–46	...	47–50	45–47	52–56	47–50	...	...	50–52	...	44–47	46–49

[a] Glyco Products Co., Inc.
[b] Atlas Powder Co.

the melted base, while water-insoluble medicinals were previously levigated with some of the melted base to a fine paste before incorporation into the remaining base. The suppository mass with the included medicinal was then spread over a flat glass surface to allow maximum surface exposure to atmosphere. After forty-eight hours, the suppository base was examined macroscopically for separation of medicinal, discoloration, lumping, liquefaction, or developed odor. The following materials were found to be compatible with the base: aspirin, 16%; Argyrol, 1%; belladonna extract, 3%; benzocaine, 1%; camphor, 1%; coal tar, 5%; Ichthyol, 5%; merbromin, 1%; peru balsam, 5%; sulfadiazine, 25%; sulfathiazole, 25%; and thymol, 1%. Liquefaction was caused by the incorporation of 25% chloral and 25% sodium bromide.

In addition to the evaluation of the new suppository base by general spot compatibility tests, specific drugs were incorporated with the base to determine any chemical or physical loss or change on storage. Phenobarbital, tannic acid, quinine hydrochloride, and chloramphenicol were chosen for compatibility and storage tests on the newly developed suppository base. Thus barbiturate, astringent, alkaloid, and antibiotic drugs commonly found in present-day suppositories are represented. After incorporating the medicinal into the base, the product was immediately assayed for the amount of medicinal present. The suppositories were stored at 40° for two weeks and again tested for the amount of medicinal present. Analytical methods as described in the individual tests were used to determine the amount medicinal present. The results and sources of assay procedures are given in Table III.

Discussion of Table I.—For the purpose of evaluating the modified bases, the pure base was given a value of 100% for the percentage dye released, and values were calculated for the modifications with this as standard from the percentages of dye released by the modifications. The data presented here indicated that white wax, water, and Aerosol OT in combination with the tested bases resulted in high percentage medicinal release and favorable melting points. The incorporation of suppository disintegrating agents gave favorable results, but the appearance of the product was grainy and undesirable.

Discussion of Table II.—The combinations of modifiers and selected bases suggested in Table I

TABLE II.—THE PERCENTAGE MEDICINAL RELEASE FROM SOME CARBOWAX AND POLYOXYETHYLENE STEARATE BASES COMPARED WITH THEOBROMA OIL

Base	Melting-Point Range, ° C.	Percentage Medicinal Release (Minutes)					
		1	2	3	5	7	9
Carbowax 4000 monostearate with 1% white wax, 10% water, and 0.5% Aerosol OT	52–54	4.2	9.2	19.2	29.5	36.7	56.8
Polyoxyethylene 30 stearate with 3% white wax, 5% water, and 0.5% Aerosol OT	49–51	2.2	8.3	10.9	30.1	88.2	100.0
Polyoxyethylene 40 stearate with 5% white wax, 10% water, and 0.5% Aerosol OT	50–52	1.2	6.1	12.5	24.2	33.4	45.5
Theobroma oil	30–35	10.7	22.1	36.4	37.4	40.7	44.1

were prepared, and the percentage medicinal release was tested over a nine-minute time interval. The data indicated that polyoxyethylene 30 stearate with 3% white wax, 5% water, and 0.5% Aerosol OT had the best medicinal release percentage as compared with the other combinations or theobroma oil. The melting-point range of this combination was in the 50° range.

Discussion of Table III.—The specific compatibility and storage tests showed that the new suppository base investigated was compatible with the medicinals of therapeutic class substances as astringent, alkaloid, barbiturate, or antibiotic.

TABLE III.—ASSAY OF MEDICINALS IN SUPPOSITORY BASES CONSISTING OF POLYOXYETHYLENE 30 STEARATE, WATER, WHITE WAX, AND AEROSOL OT IMMEDIATELY AFTER MANUFACTURE AND AFTER TWO WEEKS STORAGE AT ELEVATED TEMPERATURE

Medicament	Amount of Drug Incorporated in each Suppository, Mg.	Analytical Assay		
		Amount Medicinal Present Immediately After Manufacture, Mg.	Amount Medicinal Present After Storage at 40° for Two Weeks, Mg.	Loss of Drug, %
Phenobarbital[a]	92	92.1	91.7	0.4
Quinine hydrochloride[b]	530	530.2	527.8	0.5
Tannic acid[c]	190	190.2	188.4	0.1
Chloramphenicol[d,e]	50	49.8	49.7	0.2

[a] U. S. P. XIV, p. 454.
[b] U. S. P. XIV, p. 507.
[c] A. O. A. C., see reference (6).
[d] U. S. P. XIV, p. 130.
[e] Furnished by Parke, Davis & Co.

DISCUSSION OF RESULTS

A new water-soluble, high melting-point suppository base with a short time interval, high percentage medicinal release has been developed. This new base consists of polyoxyethylene 30 stearate-(Myrj 51), water, white wax, and Aerosol OT. The percentage wax necessary to maintain the 50° melting-point range must be varied according to the physical and chemical nature of the incorporated medicinal. Experimentation with various medicinals indicated that between the limits of 3 to 15% white wax necessary to maintain the 50° melting range, there was little effect upon the promptness of release of medicinal from the base.

The percentage of water included aids in the preparation of a product with suitable luster and smoothness thus lending pharmaceutical elegance. In incorporating water-soluble medicinals, the water provided in the formula may be utilized to predissolve the drug before incorporation into the remainder of the base.

Polyoxyethylene 30 stearate is in itself a surface-active agent; however, the use of the highly surface-active colloidal Aerosol OT in conjunction with this base substance seems to lend synergism to the surface activity and thus aids in the rapid solution of the suppository.

The bacteriological testing of modified polyoxyethylene 30 stearate showed that the substance would apparently not support microbial growth. Aerobic and anaerobic tests, air exposed plates, and plates inoculated with molds, yeasts, and Gram-positive and Gram-negative bacteria showed no microbial growth under macroscopic and microscopic observation.

Under the experimental conditions of the colorimetric test used to determine the relative values of suppository bases as to their medicinal release, 100% medicinal release was possible in nine minutes with this new base. The suppository completely dissolved. This desirable feature coupled with the fact that the developed suppository base has a melting range approximating 50° provides a water-soluble suppository base with properties suitable for shelf storage, shipping, and handling at elevated temperatures. The necessity for the base to melt at body temperature is obviated by the rapidity of solution of the dosage form.

Because of the higher melting point of the developed base, it may be formed by hand without liquefaction. Therefore, the extemporaneous preparation of suppositories in the drugstore is feasible and accomplished with less difficulty in warm weather than would be possible with a lower melting point base such as theobroma oil.

The developed suppository base is white, has a slight characteristic odor, and is a waxy solid at room temperature. The base substance is fairly inert chemically and thus presents few incompatibilities with medicinals commonly administered as suppositories. The nonionic nature of the substance aids in the general compatibility. Substances which in high percentages exhibit the marked effect of lowering the melting point of mixtures tend to cause eutectic phenomena, but this incompatibility occurs only with high concentrations of medicinals as chloral. Extremely hygroscopic salts, as sodium bromide, may cause liquefaction of the base by absorption of water from the atmosphere. Generally, the base appears to be compatible with most medicinals used in suppositories.

The compatibility and storage tests carried out with specific drugs in the therapeutic classification: astringent, alkaloid, antibiotic, and barbiturate seem to indicate a long possible shelf life for medicinal suppositories made with this base. Storage of the prepared medicinal suppositories at elevated temperatures resulted in a loss of drug so minute that it was considered to be due to experimental error in performing the respective assays. A larger percentage experimental error occurred in the gravimetric assays than did in the photometric analyses. Assay procedures were devised for drugs in the above therapeutic classifications by considering the physical and chemical properties of the base and the medicinal, then adapting these properties to an analytical procedure.

Polyoxyethylene 30 stearate has been shown to be pharmacologically nontoxic (7). This substance is at present in use in the food, drug, and cosmetic industries.

SUMMARY

1. A new water-soluble suppository base has been developed from a mixture of polyoxyethylene 30 stearate, water, white wax, and Aerosol OT.

2. The new suppository base allows a complete release of medicinal in a short time interval, yet has a high melting point which allows handling and storage at elevated temperatures.

3. The suppository base has been investigated for compatibility with medicinals, percentage medicinal release, storage capacity, and melting-point range. The tests indicate its general compatibility and the feasibility of its adoption as a suppository base.

4. The suppository base is nontoxic, relatively nonirritating, and safe for internal administration.

REFERENCES

(1) Gross, H. M., and Becker, C. H., THIS JOURNAL, **42**, 90(1953).
(2) Gross, H. M., and Becker, C. H., *ibid.*, **42**, 96(1953).
(3) "United States Pharmacopeia," Fourteenth Revision, Mack Publishing Company, Easton, Pa., 1950, p. 734.
(4) Gross, H. M., and Becker, C. H., THIS JOURNAL, **41**, 157(1952).
(5) "Atlas Surface Active Agents," Atlas Powder Company, Wilmington, Delaware, 1950, p. 10.
(6) "Methods of Analysis, A. O. A. C." 7th ed., Association of Official Agricultural Chemists, Washington, D. C., 1950, p. 134.
(7) "Feeding Tests and Auxiliary Studies on Sorbitol, Mannitol, and Four Types of Non-Ionic Emulsifiers Derived from Long Chain Fat-Forming Fatty Acids," Atlas Powder Company, Wilmington, Delaware, 1952, pp. 31–40.

A Study of the Comparative Absorption of Estrone From a Hydrophobic and a Hydrophilic Ointment Base*

by RUTH KROEGER†, GLEN J. SPERANDIO, and GLENN L. JENKINS‡

A study was made of the comparative absorption of estrone by the intact skin of white rats from a hydrophylic ointment base and a hydrophylic base, by a vaginal smear method and a uterine weight method. The results of the vaginal smear study indicate that absorption of estrone was obtained from both types of bases, but the results of the uterine weight study give conflicting answers.

THE MECHANISM of the absorption of drugs from ointment bases has been a controversial subject for many years, and numerous theories have been offered to explain the phenomena of penetration and absorption of substances from different bases. In this study the absorption of estrone by the intact skin from two different type ointment bases was compared.

From 1937 to 1946 several studies of absorption of estrogens from various liquid vehicles were reported (1–4). Moore, *et al.* (5), reported a study of absorption of estrogens from a commercial cream using the vaginal smear technique and Salmon (6) conducted human studies using this technique. Baer (7) reported faster absorption of estrone from lanolin than from petroleum jelly, and MacBryde (8) reported positive absorption of estradiol by human females from hydrous wool fat and petroleum jelly bases.

Sekanoue (9) reported the minimum effective dose for absorption was slightly smaller for water-in-oil emulsion ointments than for injections in olive oil, and Klarmann (10) stated that absorption was not more than 40 per cent, which gives only the skin effects. Goldzieher (11) made a histological study of hormone creams, and portions of these results were confirmed by

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Speert (12). These reports led to the conclusion that estrone could be used as a basis for a statistical study of absorption through the rat skin from different ointment bases.

In 1917, Stockard and Papanicolaou (13) reported vaginal cornification during the estrus cycle in guinea pigs and in 1923 Allen and Doisy (14) reported this phenomenon in rodents. It is generally agreed that the absence of leucocytes and the presence of cornified cells or a mixture of cornified and nucleated cells is an indication of estrus (15). This and the determination of uterine weight of castrated female rodents have been the basis of estrogen assays.

EXPERIMENTAL

Techniques.—Preliminary studies established the techniques used in the two major absorption studies. Female albino rats (Wistar strain, Purdue University) were ovariectomized at the age of thirty-one days and allowed to rest for ten days. Subcutaneous injections of 0.005 mg. of estrone suspension were then given on age day forty-one, followed by 0.01 mg. on age day forty-four to effect opening of the vaginal orifice for smearing.

Smears were obtained by inserting medicine droppers drawn out to a fine tip into the vagina of the rat, and gently injecting and withdrawing one drop of distilled water. The collected material was transferred to a microscope slide, dried and stained with Giemsa stain. Smears in which the majority of the cells were nonnucleated squamous cells with no leucocytes present, or nucleated and nonnucleated squamous cells were interpreted as positive. Smears containing a majority of nucleated cells, with or without leucocytes, were considered negative.

A 4-cm.^{2} area, clipped free of hair, on the dorsal side of the rat posterior to the front legs was used for ointment application. The ointment was protected on this area by means of a bandage, 2 inches by 2 inches, composed of two layers of gauze separated by a layer of waxed paper and sewn shut around the edges. The ointment was weighed directly onto the bandage and smoothed out evenly over a 2-cm.^{2} area marked in the center of the bandage. This bandage was held in place with one-quarter inch strips of adhesive tape, backed with gauze where contact was made with the rat's hair, and passed around the rat's body over the top and bottom edge of the bandage.

For determination of uterine weights, the animals were sacrificed using chloroform, and the uteri removed by separation from the mesenteric attachment and severance at the cervical junction. Each uterus was placed on moistened absorbent toweling, the mesenteric fragments removed, the horns slit longitudinally and the lumen blotted with absorbent toweling. It was then immediately placed in a tared glass cup and weighed on a damped chainomatic balance to determine the weight. The cup was then placed in a 30 cc. covered beaker and placed in a vacuum oven at 50° and 29 lb. of vacuum for twenty-four hours, after which the dry uterine weight was determined.

Ointments.—The hydrophobic base used in this study was rose water ointment, U. S. P. XIV and the hydrophilic base was hydrophilic ointment, U. S. P. XIV, both being prepared by the official procedure. In the statistical data these are referred to as A_0 and B_0, respectively. The estrone ointments contained 0.002, 0.006, and 0.01 mg. per 0.5 Gm. of each base and are designated as A_1, A_2, A_3, and B_1, B_2 and B_3, respectively.

Vaginal Smear Study.—This consisted of a crossover type study using two groups of ten rats each for each type ointment base. Each rat received four treatments consisting of the ointment base and the respective three estrone ointment concentrations. Treatments were randomized according to the table of random numbers from Fisher and Yates (16). The ovariectomized animals were segregated and assembled on age day fifty-three, litter mates and weight being evenly distributed between the two groups. Treatments were started on age day fifty four when 0.5 Gm. of the appropriate ointment was applied. Smears were taken every twelve hours until estrus was present for two successive smears. The bandage and any excess ointment were then removed and the rats allowed to rest for fourteen days before the next treatment. Smears were made once daily during rest periods to check on completeness of ovariectomy. Every rat in each group received treatment with each of the bases and estrone ointment concentrations.

Uterine Weight Study.—This consisted of five replications each containing ten randomized rats. The rats were ovariectomized, injected, and segregated as previously described. Eight rats in each replication received daily applications of 0.5 Gm. of the assigned ointment while the remaining two rats were maintained as controls to which no ointment was applied. The rats received daily treatments for ten or twenty days, were sacrificed on the eleventh and twenty-first days, respectively, and wet and dry uterine weights were determined. Six rats were reserved for zero time controls and were sacrificed on age day fifty-four without any treatment other than ovariectomy and injection.

RESULTS

Vaginal Smear Study.—The results of the interpretations of the vaginal smears obtained from the treatments under this study are shown in Figs. 1, 2, and 3. The graphs illustrate the integrated percentage responses for each twelve-hour period for the three different concentrations of estrone ointments. There was little difference between the absorption from these two bases and since there was little variability within each ointment, the estrus dose 50% was not estimated on this basis. The ten rats for each ointment base showed 100% estrus within forty-eight hours for the 0.002 mg./0.5 Gm. concentration, thirty-six hours for the 0.006 mg./0.5 Gm. concentration and twenty-four hours for the 0.1 mg./0.5 Gm. concentration. The Bliss method for probits (17), estimating probits for 0 and 100% according to the method of Miller and Tainter (18), was used to estimate the estrus dose 50% for the absorption of estrone from each base within twenty-four hours. The slopes of the regres-

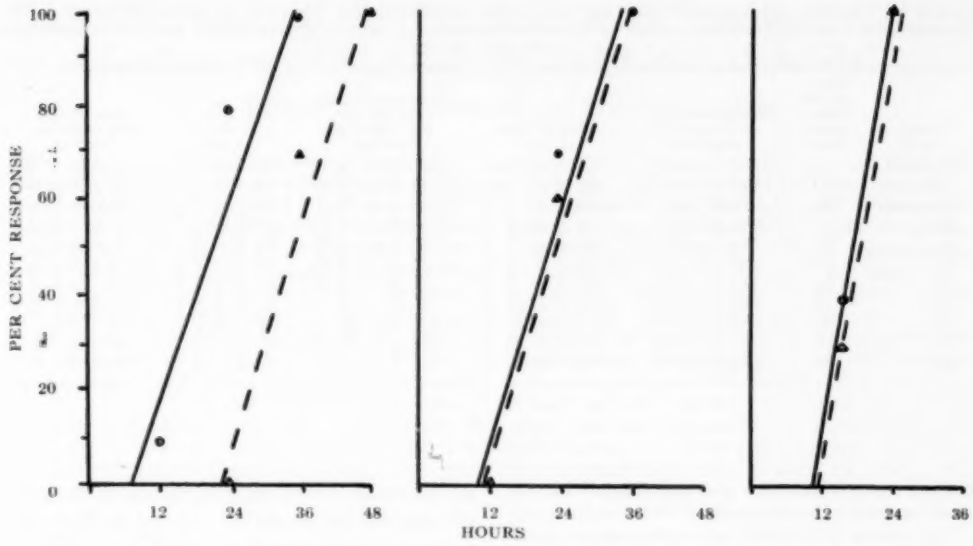

Fig. 1.—0.002 mg. Fig. 2.—0.006 mg. Fig. 3.—0.01 mg.

Per cent positive smears obtained by application of the indicated amount of estrone per 0.5 Gm. of ointment to ovariectomized rats *vs.* hours. —— rose water ointment. - - - - hydrophilic ointment

sion lines for the hydrophobic and hydrophilic ointments were estimated to be 4.5 and 4.2, respectively. The chi square test for these lines did not lead to any suspicion of nonlinearity and the estrus dose 50% for the hydrophobic base was calculated to be 0.0041 mg./0.5 Gm. of hydrophobic ointment and 0.0045 mg./0.5 Gm. of hydrophilic ointment with fiducial limits of 0.002 to 0.0081 mg./0.5 Gm. and 0.0023 to 0.0085 mg./0.5 Gm. of ointment, respectively. Since the test for parallelism showed no evidence that the lines were not parallel and there was an extreme overlap of fiducial limits, it was concluded that there was no significant difference in the absorption of estrone in these two groups of rats.

Uterine Weight Study.—Means and standard errors for the animal weights and uterine weights for the zero time controls and the five treatment replications are shown in Table I. Analyses of variance based on wet weight of uteri and dry weight of uteri expressed as mg./100 Gm. of rat indicated that the differences among the five treatment means were highly significant at the 1% level. However, the difference between the two estrone ointments was not significant. Hence when the variable, mg. of uterus weight/100 Gm. of rat, was used in the analysis, there was no evidence against the hypothesis that the rate of absorption from the two estrone ointments was equal.

If the rate of absorption of estrone is measured by the increase in the weight of the uterus, and the variable, mg. of uterus weight/100 Gm. of rat, is used in the analysis, the following assumptions are made: (*a*) The relationship between uterine weight and rat weight is linear for all weights of rats and the straight line representing this relationship passes through the origin; and (*b*) the variable, mg. of uterus weight/100 Gm. of rat, is normally distributed before tests of significance may be performed. With such a limited range on the weight of rats in this experiment, it is most difficult to investigate the validity of the first assumption. Information from previous experiments indicates that both variables, uterine weight and rat weight, are normally distributed. If this is true, then the ratio of these two variables is not normally distributed. Hence there is doubt of the validity of the second assumption.

A more statistically sound basis for analysis of these data is the analysis of covariance, where uterine weight is the variable being investigated and rat weight is the covariant whose effect is to be removed from uterine weight. In the analysis of covariance a linear relationship between the two variables is assumed for the range of rat weights in this experiment only and the tests of significance require that the variable, wet uterine weight, is normally distributed. When the analysis of covariance was used, the significant difference test, *t* test, revealed that there was significant difference between the two estrone ointments. Since the mean wet uterus weight obtained when the rose water ointment was used was greater than the weight obtained when the hydrophilic ointment was used, it would be concluded that the absorption of estrone was greater from the hydrophobic base, rose water ointment, than from the hydrophilic base, hydrophilic ointment for these rats.

DISCUSSION

Some difficulties were experienced in this study. First, it was impossible to determine whether clipping the hair of the rat influenced absorption Since, however, depilatories and shaving gave

TABLE I.—MEANS AND STANDARD ERRORS FOR ANIMAL WEIGHTS AND UTERINE WEIGHTS OBTAINED WITH TREATMENTS FOR THE DETERMINATION OF ESTRONE ABSORPTION FROM A HYDROPHOBIC AND A HYDROPHILIC OINTMENT BASE

Treatment	Duration of Treatment, Days	Weight of Rats in Grams at Sacrifice	Uterine Weights Wet Weight Mg.	Uterine Weights Wet Weight Mg./100 Gm. of Rat	Uterine Weights Dry Weight Mg.	Uterine Weights Dry Weight Mg./100 Gm. of Rat
Control	0	135.7 ± 4.2	62.6 ± 5.6	46.0 ± 3.7	17.0 ± 1.7	12.5 ± 1.2
Control	10	167.6 ± 4.0	58.1 ± 5.5	34.5 ± 2.5	16.0 ± 1.9	9.5 ± 0.9
Control	20	178.8 ± 9.0	49.6 ± 2.1	28.1 ± 2.2	12.2 ± 1.0	6.9 ± 0.8
A_0	10	163.8 ± 5.7	64.8 ± 9.7	39.3 ± 5.1	17.9 ± 4.0	10.9 ± 2.2
A_0	20	185.7 ± 6.7	56.0 ± 7.1	30.1 ± 3.6	14.3 ± 2.4	7.6 ± 1.2
B_0	10	166.4 ± 4.7	60.5 ± 4.3	36.4 ± 2.8	17.6 ± 2.6	9.4 ± 1.5
B_0	20	192.1 ± 7.0	47.7 ± 2.3	24.9 ± 1.2	11.7 ± 0.9	6.1 ± 0.5
A_2	10	156.3 ± 5.3	213.7 ± 23.7	136.6 ± 14.2	41.9 ± 4.0	26.8 ± 2.3
A_2	20	182.6 ± 5.9	274.8 ± 27.6	153.0 ± 20.9	55.5 ± 4.7	30.7 ± 3.7
B_2	10	154.4 ± 3.3	181.9 ± 25.8	118.1 ± 34.9	38.7 ± 5.1	25.0 ± 3.1
B_2	20	174.0 ± 5.7	232.8 ± 12.1	133.9 ± 6.5	49.0 ± 3.1	28.2 ± 1.7

definite signs of irritation and clipping did not, the latter seemed to be the method of choice.

The results of the study by vaginal smears would have been more valid statistically had it been possible to run an extremely large number of groups of rats and stagger the time of smearing so that percentage responses could be ascertained for other times. Likewise, the study would be enhanced if it were possible to grade the smear responses. This was not possible, however, since the number of cells constituting a smear cannot be standardized. Ratios of the cells in the microscopic field cannot be used because of the uneven distribution of the cells within a smear. The interpretations had to be made as either positive or negative after careful examination of the entire smear.

It is strongly believed that the effect of estrone observed in these studies was by absorption only, since all precautions were taken to eliminate ingestion of the ointment. The area chosen for application of the estrone was not readily accessible to the rat, the bandage prevented scratching the area and subsequent ingestion by licking the feet, and the rats were conditioned to the bandage and housed individually.

SUMMARY AND CONCLUSIONS

1. The comparative absorption of estrone by the intact skin of white rats from a hydrophobic ointment base and a hydrophilic base has been studied by a vaginal smear method and uterine weight method.

2. Results from the vaginal smear study indicate that absorption of estrone was obtained from both type of bases and that estrone was absorbed more rapidly or in larger quantities from the bases having the highest concentration of the hormone.

3. Results from the uterine weight study, when analyzed statistically, give conflicting answers:

(*a*) When the variable mg. of uterus weight/100 Gm. of rat is used, the significance tests, *t* tests, in both the wet weight and the dry weight of uteri showed no significant difference between the two estrone ointments. Hence if all the assumptions are fulfilled, it would be concluded that there was no difference between the absorption of estrone from the hydrophobic or the hydrophilic base.

(*b*) Significant differences, *t* tests, based on the adjusted treatment means obtained in the analysis of covariance indicated a significant difference between the two estrone ointments. From this it would be concluded that the absorption of estrone was better from the hydrophobic base, rose water ointment, U. S. P. XIV., than from the hydrophilic base, hydrophilic ointment, U. S. P. XIV.

4. It is believed by the authors that the results obtained in the analysis of covariance are more accurate and hence absorption of estrone is better from the hydrophobic base.

REFERENCES

(1) Loeser, A. J., *J. Obstet. Gynaecol. Brit. Empire*, **44**, 710(1937).
(2) Zondek, B., *Lancet*, **1938 I**, 1170(1938).
(3) Emmens, C. W., *J. Endocrinol.*, **2**, 368(1941).
(4) Valette, G., and Cavier, R., *Compt. rend. soc. biol.*, **140**, 255(1946).
(5) Moore, C. R., Lamar, J. K., and Beck, N., *J. Am. Med. Assoc.*, **111**, 11(1938).
(6) Salmon, U. J., *Proc. Soc. Exptl. Biol. Med.*, **38**, 481 (1938).
(7) Baer, H. I., *J. Investigative Dermatol.*, **2**, 15(1939).
(8) MacBryde, C. M., *J. Am. Med. Assoc.*, **112**, 1045 (1939).
(9) Sekanoue, K., *Japan. J. Obstet. Gynecol.*, **22**, 100 (1939); through *Chem. Abstr.*, **36**, 526(1942).
(10) Klarmann, E. G., *Am. Perfumer*, **48**, No. 11, 41 (1946).
(11) Goldzieher, M. A., *Geriatrics*, **1**, 226(1946).
(12) Speert, H., *Physiol. Revs.*, **28**, 23(1948).
(13) Stockard, C. R., and Papanicolaou, G. N., *Am. J. Anat.*, **22**, 225(1917).
(14) Allen, E., and Doisy, E. A., *J. Am. Med. Assoc.*, **81**, 819(1923).
(15) Thayer, S. A., "Vitamins and Hormones," Academic Press Inc., New York, Vol. IV, 1946, pp. 312–361.
(16) Fisher, R. A., and Yates, F., "Statistical Tables," ed. 3, Hafner Publishing Co., Inc., New York, 1948, Table of Random Numbers.
(17) Bliss, C., *Quart. J. Pharm. Pharmacol.*, **11**, 192 (1938).
(18) Miller, L. C., and Tainter, M. L., *Proc. Soc. Exptl. Biol. Med.*, **57**, 261(1944).

A Note on the Preparation of Powdered Carboxymethylcellulose and Linseed Acids*

By JOSEPH V. SWINTOSKY† and LLOYD KENNON

THOUGH THE use of acid gums in pharmaceutical technology has not been extensive, the recent literature contains references (1–6) alluding to their preparation, properties, and possible pharmaceutical applications. In the course of our studies concerned with the use of such products in pharmacy, two new powdered acid gums were prepared, namely, carboxymethylcellulose (CMC acid) and linseed acid. The literature alludes to water insensitive films cast from water solutions of CMC acid, which are prepared by ion exchange methods (7), but does not describe the preparation of a water dispersible powdered CMC acid. Also, there is described the preparation of a fibrous linseed acid (8), but not a readily dispersible powdered product. This communication describes a new procedure for preparation of these acids in a powdered, water-dispersible form.

Preparation of Powdered CMC Acid.—The CMC acid solution was prepared by running a 1% water solution of the sodium salt (CMC, Type 70, Hercules cellulose gum, premium low viscosity) through an acid activated Amberlite IR-120 resin ion exchange column. By using this method, about 80 L. of the CMC acid solution were prepared. This solution was then sprayed through an atomizing head into a 230° chamber of a Bowen Spray Drier (1). A white powder, very similar to cornstarch in appearance, was produced.

Preparation of Powdered Linseed Acid.—Fibrous linseed acid was prepared according to a method described by Anderson and Lowe (8) by extraction of clean flaxseed with a hydrochloric acid acidified aqueous solution. The mucilaginous solution derived from this extraction was treated with ethyl alcohol to precipitate the acid gum. In two such consecutive precipitations a product was obtained relatively free from hydrochloric acid and other impurities. This fibrous product was white and like paper pulp in appearance, but on dissolution in water a tan solution resulted. A 2% aqueous solution of the linseed acid was passed through the ion exchange column in a manner identical to that employed in the preparation of the CMC acid solution. This was done in an attempt to remove any metal ions still remaining in the acid gum after the alcohol precipitation. The tan colored solution so obtained was then spray dried at about 230°, and a stable tan powdered linseed acid was obtained.

Properties of the Powdered Acid Gums.—Ash determination, acid equivalent weight determinations, dispersibility studies, and *p*H measurements were made for each of these powdered acids.

For CMC acid the average ash content was found to be 0.78%. The average acid equivalent weight was 287. The product was readily dispersible in water forming cloudy, rapidly hydrated dispersions. In organic solvents the degree of dispersibility dropped off with decreasing polarity of the solvent, being somewhat dispersible in a liquid like isopropyl alcohol, and being clumpy and practically unsuspendable in hydrocarbons. A 0.5% dispersion in water showed a *p*H of about 2.3; and alkali titration of this dispersion yielded a potentiometric titration curve typical of a moderately strong monobasic acid. The single inflection curve was very similar to that observed for arabic acid (1) and related acid gums. Preliminary studies indicate that this powdered acid gum possesses tablet disintegrant properties analogous to that of powdered alginic acid (6).

For linseed acid the average ash content was found to be 0.67%. The average acid equivalent weight was 757. The dispersibility characteristics were similar to those exhibited by CMC acid, except that in water a considerably greater time was required for hydration and dispersion; and the acid gum showed a greater tendency to clump when placed in water. A 1.5% dispersion in water showed a *p*H of about 1.7; and alkali titration of this solution yielded a potentiometric titration curve very similar in character to that observed for the CMC acid.

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REFERENCES

(1) Schleif, R. H., Higuchi, T., and Busse, L. W., THIS JOURNAL, **40**, 98, 221(1951).
(2) "Kelacid, A Kelco Algin Product," a brochure of the Kelco Co., Chicago.
(3) Nilson, H. W., and Lemon, J. M., U. S. Dept. of Interior, Fish and Wildlife Service, Research Report No. 4 (1942).
(4) Nilson, H. W., and Wagner, J. A., *Proc. Soc. Exptl., Biol. Med.*, **76**, 630(1951).
(5) Ouer, R. A., *Ann. Allergy*, **7**, 681(1949).
(6) Berry, H., and Ridout, C. W., *J. Pharm. Pharmacol.*, **2**, 619(1950).
(7) "Cellulose Gum Free Acid," a brochure of the Hercules Powder Co., Wilmington, Del.
(8) Anderson, E., and Lowe, H. J., *J. Biol. Chem.*, **168**, 289(1947).

Book Notices

Organic Reactions. Vol. VII. ROGER ADAMS, Editor-in-Chief. John Wiley & Sons, Inc., New York and London, 1953. vii + 440 pp. 16 x 23.5 cm. Price $9.

This book is the seventh volume of a series started in 1942, and designed to provide a compilation of all the more important synthetic chemical reactions. As in previous volumes, each chapter is written by an author who has had special experience with the reaction about which he is writing.

The reactions covered in this volume include the Pechmann Reaction, the Skraup Synthesis of Quinolines, Carbon-Carbon Alkylations with Amines and Ammonium Salts, the von Braun Cyanogen Bromide Reaction, Hydrogenolysis of Benzyl Groups attached to Oxygen, Nitrogen, or Sulfur; the Nitrosation of Aliphatic Carbon Atoms, and Epoxidation and Hydroxylation of Ethylenic Compounds with Organic Peracids.

Each chapter in the series presents a comprehensive survey of the reaction under consideration. The survey includes a general discussion of method, modifications, examples of applicability, special precautions, a detailed description of the procedure, expected yields, and other pertinent data. Tables are included in each chapter listing compounds which have been prepared by or subjected to the reaction under discussion in that chapter. Among the contributors to Volume VII of *Organic Reactions* are Walter H. Hartung, Professor of Pharmaceutical Chemistry in the College of Pharmacy at the University of North Carolina, and Robert Simonoff, of the William R. Rorer Co., Inc., who furnished the chapter on the hydrogenolysis of benzyl groups attached to oxygen, nitrogen, or sulfur. The entire book is thoroughly documented with references to the original literature and is provided with a satisfactory index. The book should be of special interest to anyone engaged in research involving the syntheses of organic compounds.

Essentials of Physiological Chemistry. 4th ed. By ARTHUR K. ANDERSON. John Wiley & Sons, Inc., New York, (Chapman and Hall, Ltd., London), 1953. vii + 480 pp. 15.5 x 23.5 cm. Price $5.

This book by Arthur K. Anderson, who is Professor of Physiological Chemistry at the Pennsylvania State College, is now in its fourth edition, the first edition having been published in 1935. The book is designed to present the more important facts of biochemistry as related to the animal body in a form which will be understandable to the student with only a limited preparation in chemistry and biochemistry, and is designed especially for undergraduate students in biochemistry, home economics, premedicine, chemistry, bacteriology, and agriculture. Many advances which have occurred since the appearance of the third edition in 1947 will be found in the new edition. Among some of the more important changes are the inclusion of a section on isotopes in the chapter on biophysical chemistry; an expansion of the chapter on lipids and antioxidants; and more or less completely rewritten chapters on enzymes and carbohydrate metabolism including recent developments. The subject of chemotherapy and adrenal hormones has been thoroughly revised, and in the chapter on vitamins more emphasis than heretofore has been placed on their physiological action.

Synthetic Organic Chemistry. By ROMEO B. WAGNER and HARRY D. ZOOK. John Wiley & Sons, Inc., New York, 1953. 15.5 x 23.5 cm. xii + 885 pp. Price $11.50.

In this book methods of organic syntheses most frequently employed in the preparation of mono- and di-functional compounds are summarized. The methods described are collected in chapters each of which covers the formation of compounds containing a particular functional group or related groups. No attempt has been made to present detailed discussions, but references to the original literature and to other books and review articles are given for the better preparative procedures. The text is supplemented by tables of additional references and other examples which constitute a convenient index to the literature relating to the preparation of starting materials of relatively simple structure.

In compiling *Synthetic Organic Chemistry*, the authors have reviewed the more important books and journals relating to organic syntheses which were published between 1918 and 1951. The book is provided with an index which is exceptionally complete and which emphasizes particularly the reactions of organic compounds. It is quite unusual for any book to include so much information in a relatively small amount of space.

The compounds are classified according to functional groups and are arranged according to their carbon content into aliphatic, alicyclic, aromatic, and heterocyclic series. The book deserves wide acceptance by all who are interested in synthetic organic chemistry, either from a general or pharmaceutical viewpoint.

Condensed Pyridazine and Pyrazine Rings (Cinnolines, Phthalazines, and Quinoxalines). By J. C. E. SIMPSON. Interscience Publishers, Inc., New York, 1953. xvi + 394 pp. 16.5 x 24 cm. Price $12.50. (Subscription Price $11.25).

This is the fifth volume of a series of monographs covering heterocyclic chemistry in a comprehensive manner. In this, as in the earlier volumes, the significance and interrelations of the various compounds are stressed.

In the fifth volume the subject matter is presented in three sections: (1) the cinnolines, (2) the phthalazines, and (3) the quinoxalines. The first two groups are formed by the condensation of a pyridazine nucleus with an aromatic ring and the third by the fusion of a pyrazine with an aromatic ring.

The monograph is written with the objective of serving as a continuation of Meyer-Jacobson's *Lehrbuch der organischen Chemie*, Volume II, 3. To avoid the creation of possible gaps, the literature has been covered from 1917 through 1949.

Technique of Organic Chemistry. Vol. VIII—Investigation of Rates and Mechanisms of Reactions. S. L. FRIESS and A. WEISSBERGER, Editors. Interscience Publishers, Inc., New York, 1953. xxiii + 760 pp. 16.5 x 24 cm. Price $12.50.

This book is the eighth in a series designed to present a comprehensive survey of the techniques used for the investigation of organic compounds. A very important phase in any research problem involving organic compounds is an investigation of the mechanism of the reactions employed. Information on the rates and mechanisms of reactions can be most valuable in directing operations and using the best conditions for synthetic or analytical work.

This volume presents in a very competent manner the theoretical and experimental procedures used by organic chemists to establish the mechanisms of reactions. Reactions in both gaseous and liquid phases including catalyzed reactions in homogeneous systems are thoroughly discussed. Polymerization and biochemical kinetics are also covered.

The scope of the book is illustrated by the titles of the 10 chapters which are as follows: General Theory of Rate Processes; Fundamental Operations and Measurements in Obtaining Rate Data; Special Experimental Methods for Determination of Rate Data; Evaluation and Interpretation of Rate Data; Homogeneous Gas Phase Reactions; Reactions in the Liquid Phase; Homogeneous Catalysis in Solution; Polymerization and Polymer Reactions; Biological Reactions; Rapid Reactions.

Each chapter is divided into several parts, and each is written by well-known specialists in the field of the subjects covered. The book is adequately documented with references to the original literature and is provided with an index to Volume VIII and abbreviated cumulative indexes to Volumes I through VIII. The *Technique of Organic Chemistry* series illustrates the application of physical chemistry to organic syntheses and constitutes one of the most important contributions to the field of organic chemistry published during recent years.

Federal Food, Drug, and Cosmetic Act—Judicial and Administrative Record, 1951–1952. By VINCENT A. KLEINFELD and CHARLES WESLEY DUNN. (Food Law Institute Series). Commerce Clearing house, Inc., Chicago, 1953. 588 pp. 17.5 x 25.5 cm Price $12.

This book follows, for the period 1951–52, the general pattern established by Kleinfeld and Dunn in their *Federal Food, Drug, and Cosmetic Act—Judicial and Administrative Record, 1938–1949*, which was reviewed in THIS JOURNAL **40,** 304(1951), and *Federal Food, Drug, and Cosmetic Act—Judicial and Administrative Record, 1949–50,* also reviewed in THIS JOURNAL **41,** 51(1952). These books, as indicated in previous reviews, are not designed to serve as textbooks, but rather as useful guides and source books which can be used to advantage either by attorneys or by laymen upon whose interests the Federal Food, Drug, and Cosmetic Act impinge.

This volume contains all opinions and decisions for the period covered and additional material not to be found elsewhere except in F. D. A. Notices of Judgment and in the Commerce Clearing House Food, Drug, Cosmetic Law Reports. In addition, significant opinions under the Federal Trade Commission Act which relate to foods, drugs, and cosmetics, are supplied. The value of the book is enhanced by a cumulative table of cases cited in the authors' earlier books as well as in this one. The attitude of the Food and Drug Administration on certain questions can often be determined by consulting the "Statements of General Policy or Interpretation," which are reproduced in full in this useful book. Important definitions and standards for food promulgated by the Federal Security Administrator which have the force and effect of law, are reproduced in full text. A suggested form of petition for review of an order defining and standardizing a food is included. As in previous editions, the value of the book is greatly enhanced by an apparently complete subject index. Several significant cases under the Food and Drug Act of 1906 not covered in previous editions, are included in the 1951–1952 book.

The advisability or necessity of reproducing in full the Federal Food, Drug and Cosmetic Act, the principal regulations, and related laws such as the Durham-Humphrey Act, as well as the Federal Trade Commission Act, requiring nearly 100 pages in the book, may be open to question. It may, however, add sufficiently to the convenience of the user of the book to be worth the space devoted to it. Undoubtedly this latest Food Law Institute publication will be widely accepted as a valuable addition to the libraries of all who are concerned with the important area of federal regulation and control of foods, drugs, therapeutic devices, and cosmetics.

General Biochemistry. By JOSEPH S. FRUTON and SOFIA SIMONDS. John Wiley & Sons, New York, 1953. 16 x 23.5 cm. xii + 940 pp. Price $10.

This book is designed to fill the need of students who wish to obtain a knowledge of the structure of modern biochemistry from a general point of view. Attention is focused on principles illustrated by data from studies of plants, microorganisms, and animals.

In the first chapter the scope and history of biochemistry is presented in an interesting and logical manner. Following the introduction the book is divided into seven parts, each of which includes three or more chapters. Part I begins with a discussion of protein chemistry and discusses the central role proteins exert in the chemical activity of living matter.

The authors proceed with discussions of the general properties of enzymes and of equilibria and rates in enzyme-catalyzed reactions. Other parts include discussions of biological oxidation and the intermediate metabolism of carbohydrates, lipids, and nitrogen compounds.

The last part of the book covers general aspects of metabolism in which the role of inorganic ions and heat changes in metabolism and the hormonal control of metabolism are followed by a discussion of vitamins and growth factors. The book is documented with pertinent references to the original literature and is provided with an adequate index.

BOOKS RECEIVED

Pharmacy Quiz Journal. 2nd ed. By JOSEPH A. ORTOLAN. Private Printing, 1953. 240 pp. 14 x 21.5 cm.

The Physician in Atomic Defense. By THAD P. SEARS. The Year Book Publishers, Inc., Chicago, 1953. 308 pp. 14.5 x 21 cm. Price $6.

Aviation–Toxicology—An Introduction to the Subject and a Handbook of Data. Prepared under the direction of the Committee on Aviation Toxicology, Aero Medical Association. The Blakiston Co., New York, 1953. 120 pp. 14.5 x 21.5 cm. Price $4.

Diuretic Therapy—The Pharmacology of Diuretic Agents and the Clinical Management of the Edematous Patient. By ALFRED VOGL, M.D. The Williams & Wilkins Co., Baltimore, 1953. 248 pp. 16 x 23.5 cm. Price $5.

Essentials of Physiological Chemistry. 4th ed. By ARTHUR K. ANDERSON. John Wiley & Sons, Inc., New York, 1953. 480 pp. 15.5 x 23.5 cm. Price $5.

The Suprarenal Cortex. J. M. YOFFEY, Editor. Proceedings of the Fifth Symposium of the Colston Research Society. Academic Press, Inc., New York, 1953. 232 pp. 19.5 x 26 cm. Price $6.80.

An Inventory of Social and Economic Research in Health. 1953 ed. Compiled by FREDERICK R. STRUNK. Health Information Foundation, New York. 180 pp. 15.3 x 23 cm.

Federal Food, Drug, and Cosmetic Act—Judicial and Administrative Record, 1951–1952. By VINCENT A. KLEINFELD and CHARLES WESLEY DUNN. Commerce Clearing House, Inc., Chicago, 1953. 588 pp. 17.5 x 25.5 cm. Price $12.

Organic Reactions. Vol. VII. ROGER ADAMS, Editor-in-Chief. John Wiley & Sons, Inc., New York, 1953. 440 pp. 16 x 23.5 cm. Price $9.

Encyclopedia of Chemical Technology. RAYMOND E. KIRK and DONALD F. OTHMER, Editors. (Vol. X: Pentacene to Polymethine Dyes.) The Interscience Encyclopedia, Inc., New York, 1953. 976 pp. 20 x 27 cm. Price $25.

Substances Naturelles de Synthèse. Vol. V. By LÉON VELLUZ. Masson et Cie, Paris, 1953. 206 pp. 16 x 23.5 cm. Price 1,500 *fr.*

Substances Naturelles de Synthèse, Préparations et Méthodes de Laboratoire. Vol. VI. LÉON VELLUZ, Editor. Masson & Cie, Paris, 1953. 156 pp. 16 x 22.5 cm.

Technique of Organic Chemistry—Investigation of Rates and Mechanism of Reactions. S. L. FRIESS and A. WEISSBERGER, Editors. Interscience Publishers, Inc., New York, 1953. 760 pp. 16.5 x 24 cm. Price $12.50.

Conferences on Drug Addiction Among Adolescents, held at the New York Academy of Medicine. The Blakiston Co., New York, 1953. 320 pp. 16 x 23.5 cm. Price $4.

Individual and Community Health. By WILLIAM W. STILES. The Blakiston Co., New York, 1953. 492 pp. 16 x 23.5 cm. Price $6.

1953 Medical Progress, a Review of Medical Advances During 1952. MORRIS FISHBEIN, Editor. The Blakiston Co., New York, 1953. 301 pp. 16 x 23.5 cm. Price $5.

Natural Child Spacing—The Body Temperature Method of Child Planning. By M. EDWARD DAVIS. Hanover House, Garden City, N. Y., 1953. 67 pp. 14 x 21 cm. Price $2.

The Natural History of Infectious Diseases. 2nd ed By SIR MACFARLANE BURNET. Cambridge University Press, New York, 1953. 356 pp. 14.5 x 22.5 cm. $4.50.

Condensed Pyridazine and Pyrazine Rings—Cinnolines, Phthalazines, and Quinoxalines. By J. C. E. SIMPSON. Interscience Publishers, Inc., New York, 1953. 394 pp. 16.5 x 24 cm. Price $12.50 (Subscription Price $11.25).

Collectanea Pharmaceutica Suecica. Vol. VII. Edited by the Library of the Royal Pharmaceutical Institute, Stockholm. Ivar Haeggstroems Boktryckeri, Stockholm, 1953. 122 pp. 15.5 x 23 cm.

Handbook of Material Trade Names. By O. T. ZIMMERMAN and IRVIN LAVINE. Industrial Research Service, Dover, N. H., 1953. xvi + 794 pp. 19.5 x 27 cm.

General Biochemistry. By JOSEPH S. FRUTON and SOFIA SIMMONDS. John Wiley & Sons, New York, 1953. xii + 940 pp. 16 x 23.5 cm. Price $10.

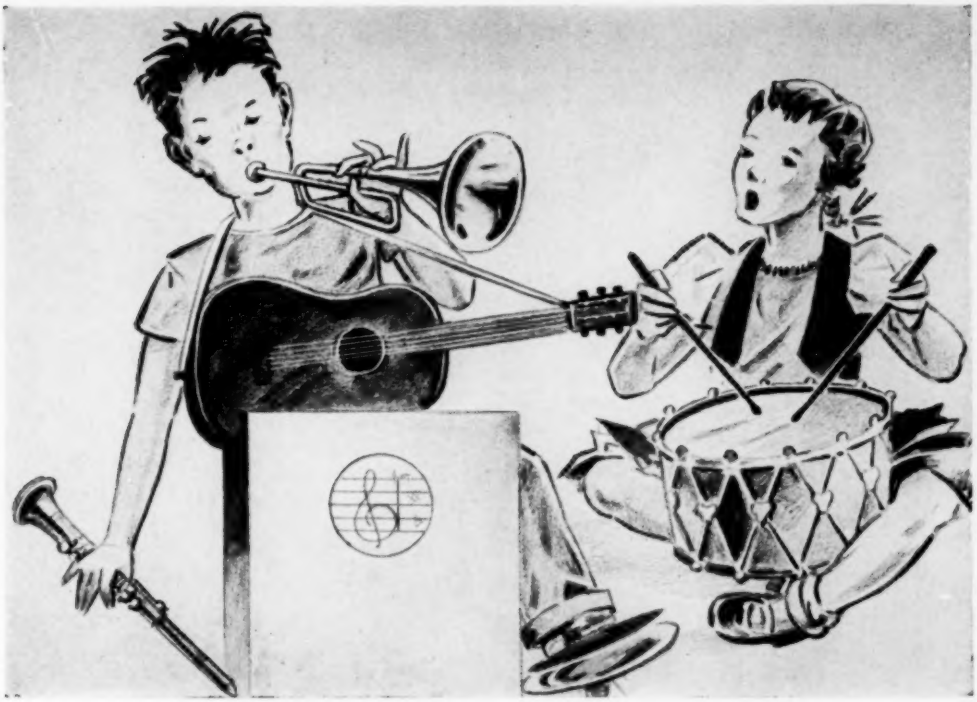
Organic Chemistry, An Advanced Treatise. Vol. III. HENRY GILMAN, Editor. John Wiley & Sons, New York, 1953. x + 580 pp. 16 x 23.5 cm. Price $8.75.

Organic Chemistry, An Advanced Treatise. Vol. IV. HENRY GILMAN, Editor. John Wiley & Sons, New York, 1953. x + 1,245 pp. 16 x 23.5 cm. Price $8.75.

Synthetic Organic Chemistry. By ROMEO B. WAGNER and HARRY D. ZOOK. John Wiley & Sons, New York, 1953. xii + 885 pp. 15.5 x 23.5 cm. Price $11.50.

Ion Exchangers in Analytical Chemistry. By OLOF SAMUELSON. John Wiley & Sons, Inc., New York, 1953. ix + 291 pp. 15.5 x 23.5 cm. Price $6.50.

Méthodes et Réactions de l'Analyse Organique—Déterminations Générales et Recherches Fonctionelles. Vol. II. LÉON VELLUZ, Editor. Masson & Cie, Paris, 1953. (Vol. II: *Méthodes de Caractérisation* by MAURICE PESEZ and PIERRE POIRIER.) 278 pp. 17 x 25.5 cm.



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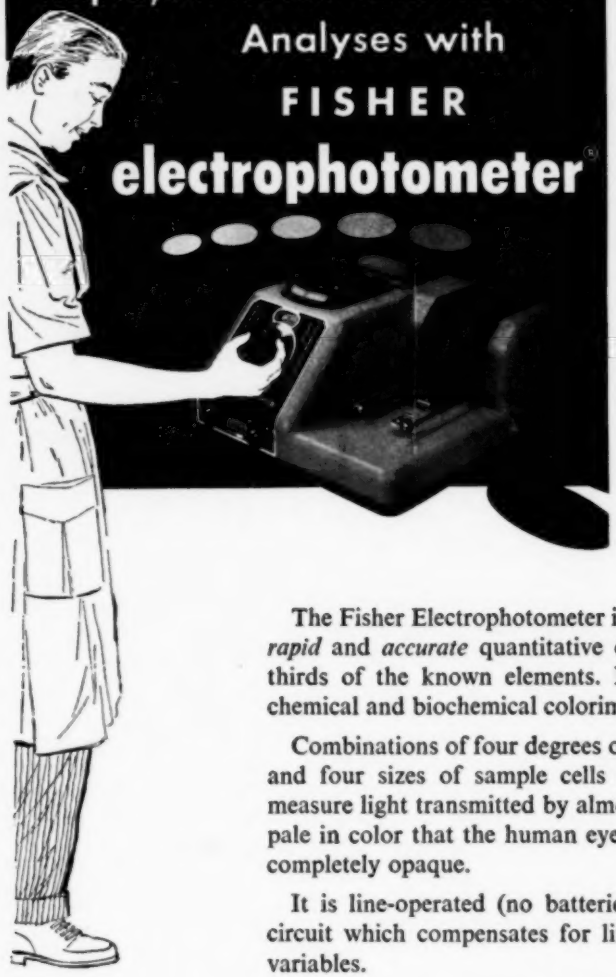

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